# SIMULTANEOUS ESTIMATION OF REPAGLINIDE AND METFORMIN HYDROCHLORIDE IN TABLET DOSAGE FORM BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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In

**PHARMACEUTICAL ANALYSIS**

**Submitted by**

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**MAY- 2012**

## CERTIFICATE

This is to certify that the dissertation entitled **"SIMULTANEOUS ESTIMATION OF REPAGLINIDE AND METFORMIN HYDROCHLORIDE IN TABLET DOSAGE FORM BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY''** is a bonafide and genuine research work carried out at Department of Pharmaceutical Analysis, K.K. College of Pharmacy, Chennai – 600122, by **Mr. R. VISWAPRASAD REDDY** during the academic year 2011-2012 under my direct guidance and supervision. This dissertation submitted in partial fulfillment for the award of the award of **Degree of Master of Pharmacy (Pharmaceutical Analysis)** to The Tamil Nadu Dr. M.G.R Medical University, Chennai – 600032.

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***R.Viswaprasad Reddy***

*DEDICATED TO*

*MY BELOVED PARENTS,*

*BROTHER, &*

*MY FRIENDS...*

# LIST OF ABBREVIATIONS USED

| | | |
|---|---|---|
| ACN | - | Acetonitrile |
| AMP | - | Adenosine mono phosphate |
| AOAC | - | Association of analytical communities |
| ASTM | - | American society for testing materials |
| ATP | - | Adenosine tri phosphate |
| AUC | - | Area under curve |
| °C | - | Degree Celsius |
| g | - | Grams |
| GLUT-4 | - | Glucose Transporter |
| HPTLC | - | High Pressure Thin Layer Chromatography |
| ICH | - | International Conference on Harmonization |
| IS | - | Internal standard |
| LOD | - | Limit of Detection |
| LOQ | - | Limit of Quantification |
| MET | - | Metformin |
| mg | - | Milligram |
| ml | - | Millilitre |
| NIDDM | - | Non Insulin dependent Diabetes mellitus |
| nm | - | Nanometer |
| ODS | - | Octadecyl silane |
| pH | - | Negative Logarithm of Hydrogen Ion |

| | | |
|---|---|---|
| $r^2$ | - | Correlation coefficient |
| RP-HPLC | - | Reverse Phase High Performance Liquid Chromatography |
| rpm | - | Rotations per Minute |
| Rt | - | Retention Time |
| S.D. | - | Standard Deviation |
| S.E. | - | Standard Error |
| USP | - | United States of Pharmacopoeia |
| USP/NF | - | United States pharmacopeia/National formulary |
| UV | - | Ultraviolet |
| v/ v | - | Volume/Volume |
| % | - | Percentage |
| % RSD | - | Percentage Relative Standard Deviation |
| µ | - | Micron |
| µl | - | Microlitre |
| λ | - | Lambda |
| µg/ ml | - | Microgram per Milliliter |

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# 1. INTRODUCTION

## 1.1 ANALYTICAL CHEMISTRY

Analytical chemistry is a branch of chemistry that deals with the separation, identification and determination of components in a sample. It is the science of making quantitative measurements, which requires background knowledge of chemical and physical concepts. The use of instruments is an exciting and fascinating part of chemical analysis that interacts with all areas of chemistry and with many other areas of pure and applied science. Analytical instruments plays an important role in the production and evaluation of new products, protection of consumers and environment, it also provides the lower detection limits required to assured safe foods, water and air[1]. Traditionally, analytical chemistry can be classified into two types

- Qualitative analysis
- Quantitative analysis

Qualitative analysis gives an indication of the identity of the chemical species in the sample and quantitative analysis determines the amount of one or more of these components[2].

**Classification of analytical techniques[3, 4]**

The analytical techniques can be classified on the basis of type of properties in the following way.

i) Chemical methods of analysis

ii) Electrical methods of analysis

iii) Optical methods of analysis

iv) Nuclear radiation methods of analysis

v) Thermal methods of analysis

vi) Separation methods

These methods can further be classified into different techniques depending on the measurement of a characteristic property based on either the nature or the amount of the desired constituent of the sample.

**i) Chemical methods of analysis**

These methods are based on the primary role of a chemical reaction. In these methods, the direct measurement of mass is carried out by one of the two procedures, i.e. by weighing or by measuring volume.

- Gravimetry
- Volumetry

**ii) Electrical methods of analysis**

An electrical method of analysis also known as electroanalytical method can be defined as one, in which an electrochemical property of a solution is measured. A classification of electroanalytical methods can be made by measuring different electrical quantities, such as, potential, current, quantity of current, resistance and dielectric constant. These methods have different names on the basis of the measurement of these quantities and are stated below.

- Potentiometry
- Amperometry
- Voltammetry
- Calorimetry

- Conductometry and High Frequency Methods

**iii) Optical methods of analysis**

These methods are now called as spectroscopic methods of analysis. In these methods the first instruments were developed for use of visible region and therefore called optical methods.

The important spectroscopic methods are mentioned below.

- Emission Spectroscopy
- Absorption Spectroscopy
- Ultraviolet and Visible Absorption Spectroscopy
- Infrared Absorption Spectroscopy
- Photofluorometry
- Turbidimetry and Nephelometry
- Raman Spectroscopy

**iv) Nuclear methods**

Some techniques which can provide analytical information based on nuclear properties. Each of these properties or combinations of them can be studied suitably by analytical chemistry. Nuclear method can be group into following.

- Radiochemical Methods
- Radiometric Methods
- Isotopic Dilution Methods
- Activation Analysis
- Mossbauer Spectroscopy
- Nuclear Magnetic Resonance Spectroscopy
- Mass Spectrometry

**v) Thermal methods of analysis**

In thermal methods of analysis some property of the system is measured as a function of temperature. In some of these methods the temperature is used as an independent variable while in some others as a dependent variable say time. The recorded curves are helpful in interpreting the thermal behaviour of the sample. Some commonly used methods are:

- Thermogravimetric Analysis (TGA)
- Derivative thermo-gravimetry (DTG)
- Differential Thermal Analysis (DTA)
- Differential Scanning Calorimetry (DSC)
- Thermometric Enthalpy Titrations (TET)

**vi) Separation methods**

In separations, in general by appropriate reactions, the desired constituent is brought into one phase and interfering elements are brought into another and the phases being separated by physical processes. Some methods of separation are the following:

A. Classical methods

- Precipitation
- Distillation
- Sublimation
- Formation of complexes

B. Modern methods

- Chromatography
- Solvent extraction
- Ion-Exchange
- Electrophoresis

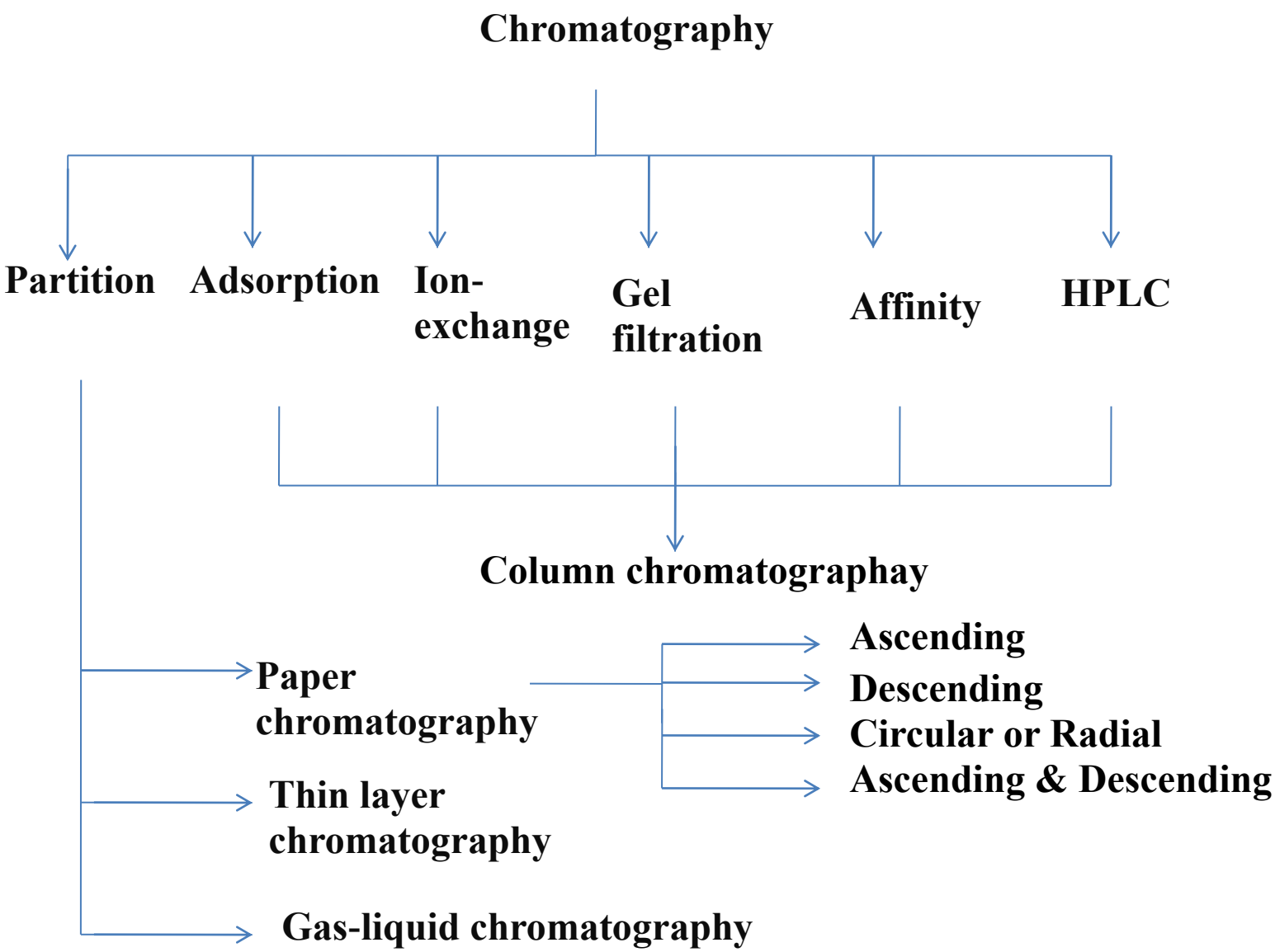
### 1.3 Important considerations in analytical methods[5]

- The instrument most visible and exciting element of the analytical method, only one component of the total analysis
- The analyst should determine the nature of the sample, the end use of the analytical results, the species to be analyzed.
- Quantitative information may include elemental composition, oxidation state, functional groups, major components, minor components, complete identification in the given sample.
- Quantitative data include accuracy and precision, range of expected analyte.
- Methods such as controlling the atmosphere to which the sample is exposed, controlling the temperature of the sample, buffering the pH of sample solutions.

### CHROMATOGRAPHY[6]

Chromatography is relatively a new technique which was first invented by M. Tswett, a botanist in 1906 in Warsaw. Chromatography is a physical separation method in which the components of a mixture are separated by differences in their distribution between two phases, one of which is stationary (stationary phase) while the other (mobile phase) moves through it in a definite direction. The substances must interact with the stationary phase to be retained and separated by it.

**CLASSIFICATION OF CHROMATOGRAPHY**




## 1.2. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY[7]

High-performance liquid chromatography (HPLC) is a form of liquid chromatography used to separate compounds that are dissolved in solution. High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. The compounds are separated

by injecting a plug of the sample mixture onto the column. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Most of the drugs in multicomponent dosage form can be analyzed by HPLC method because of the several advantages like rapidity, specificity, accuracy, precision, and ease of automation in these methods. HPLC Method eliminates tedious extraction and isolation procedures.

**Principle**

The principle involved in HPLC is separation of compounds in a mixture more efficiently and also quickly than that of traditional column chromatography. The separation of compounds is due to their relative differences in travel through the column on application of pressure exerted through mobile phase or carrying liquid. The compounds of the mixture travel with different rates due to their relative affinities with the solvent and stationary phase. Compounds with higher affinity towards stationary phase of the column travels slowly and vice-versa. The above principle is similar to that of column chromatography but in HPLC, The separation is more effective due to greater surface area achieved due to very small particle size of stationary phase in comparison to that used in column chromatography. This decrease in particle size increases has disadvantage that it proportionately enhances the flow time and run time due to increased surface area. To minimize this obstacle the high pressure is applied to the flow of mobile phase through the column by use of pumps.

All factor affecting separation on liquid column chromatography apply to this techniques also, e.g. Plate height, sample, distribution between the stationary phase and liquid phases various methods of development of the chromatograms (elution, gradient elution etc) can be used in this technique.

**Instrumentation**

The HPLC System consist of

1. A solvent reservoir and mixing system
2. A high pressure pump.
3. A sample inlet pump.
4. A column.
5. A detector and recording unit.

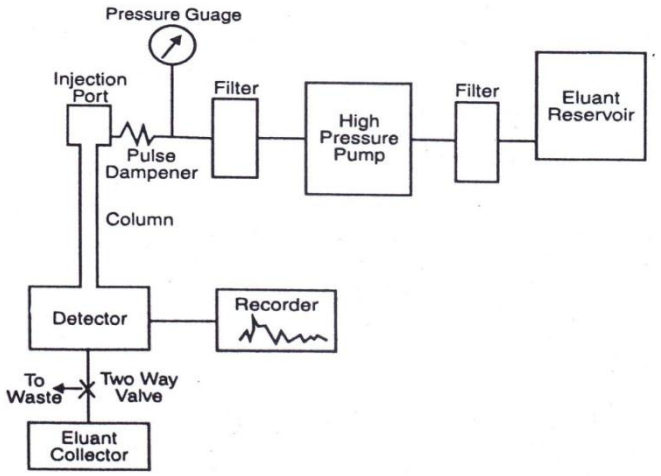



Fig: 1 – **Schematic representation of High performance liquid chromatography**

**Apparatus and materials**

1. The column.
2. Column packing
3. Column packing procedure.
4. Chromatography solvent (Mobile Phase)
5. Pumping systems.
6. Detector systems.
7. Practical procedures.

**Different modes of separations in HPLC**

**Normal Phase Mode:**

In these the stationary phase is polar and the mobile phase is non-polar in nature. In these techniques, non-polar compounds travel faster and are eluted first. This is because of the lower affinity between the non-polar compounds and the stationary phase.

**Reversed Phase Mode:**

The stationary phase is Non-polar Hydrophobic packing with Octyl or Octa decyl functional group bounded to silica gel and the mobile phase is polar solvent. An aqueous mobile phase allows the use of secondary solute chemical equilibrium to control retention and selectivity. The polar compounds gets eluted first in this mode and non-polar compounds are retained for longer times. As most of the pharmaceutical drugs are polar in nature, they are not retained for longer times and hence elute faster. The different columns used are Octa Decyl Silane ($C_{18}$), octa silane ($C_8$), tetra silane ($C_4$) etc.

**Ion Exchange Chromatography**

The stationary phase contains ionic groups like $NR_3^+$ or $SO_3^-$ which interact with the ionic groups of the sample molecules. This is suitable for the separation of charged molecules only.

**Affinity Chromatography:**

In these techniques highly specific biochemical interactions are used for separation. The stationary phase contains specific group of molecules which can absorb the sample if certain steric and charge related conditions are satisfied.

**Size Exclusion Chromatography:**

It separates molecules accordingly to their molecular mass. Largest molecules are eluted first and the smallest molecules last

**Applications**

- High pressure liquid chromatography more sensitive detectors, if promises to become more and more important. HPLC offers the advantages of speed, resolution and sensitivity. The column may be reversed. It is especially useful for separating the high molecular weight compounds which have either a two pressure or undergo pyrolysis when subjected to the higher required temperatures of has chromatography.
- The chromatography of separation of barbiturates by HPLC method.
- The wide applicability speed and selectivity of HPLC have resulted in it becoming the most popular form of chromatography virtually all type of biological molecule have been purified.
- RP-HPLC is particularly useful for the separation of the polar components such as drugs and their metabolites, peptides, vitamins, polyphenol, and steroids.

## ANALYTICAL METHOD DEVELOPMENT

Methods are developed for new products, when no official methods are available. Alternate methods for existing products are developed to reduce the cost and time for better precision and ruggedness. Trial runs are conducted, method is optimized and validated[8].

### Steps of method development[9]:

Documentation starts at the very beginning of the development process, a system for full documentation of the development studies must be established. All data relating to the studies must be recorded in laboratory notebook or electronic database.

### Analyte standard characterization:

All known information about the analyte and its structure is collected i.e., physical and chemical properties, toxicity, purity, hygroscopic nature, solubility and stability. The standard analyte (100% purity) is obtained. Necessary arrangement is made for the proper storage (refrigerator, desiccators and freezer). When multiple components are to be analyzed in the sample matrix, the number of components is noted, data is assembled and the availability of standards for each one is determined. Only those methods (MS, GC, HPLC etc.,) that are compatible with sample stability are considered.

### 2. Method requirements:

The goals or requirements of the analytical method that need to be developed are considered and the analytical figures of merit are defined. The required detection limits, selectivity, linearity, range, accuracy and precision are defined.

**3. Literature search and prior methodology:**

The literature for all type of information related to the analyte is surveyed. For physical and chemical properties, solubility and relevant analytical methods. Books, periodicals, chemical manufacturers and regulatory agency compendia such as USP/NF, AOAC and ASTM publications are convenient.

**4. Choosing a method:**

Using the information in the literatures and prints, methodology is adopted. The methods are modified wherever necessary. Sometimes it is necessary to acquire additional instrumentation to reproduce, modify, improve or validate existing methods for in-house analytes and samples.If there is no prior methods for the analyte in the literature, from analogy, the compounds that are similar in structure and chemical properties are investigated and are worked out. There is usually one compound for which analytical method already exist that is similar to the analyte of interest.

**5. Instrumental setup and initial studies:**

The required instrumentation is setup. Installation, operational and performance qualification of instrumentation using laboratory standard operating procedures (SOP's) are verified. Always new consumables (e.g. solvents, filters and gases) are used, for example, method development is never started, on a HPLC column that has been used earlier. Analyte standard in a suitable injection/ introduction solution and in known concentrations and solvents are prepared. It is important to start with an authentic, known standard rather than with a complex sample matrix. If the sample is extremely close to the standard (e.g. bulk drug), then it is possible to work with the

actual sample. Analysis is done using analytical conditions described in the existing literature.

**6. Optimization:**

During optimization one parameter is changed at a time, and set of conditions are isolated, rather than using a trial and error approach. Work has been done from an organized methodical plan, and every step is documented (in a lab notebook) in case of dead ends.

**7. Documentation of analytical figures of merit:**

The originally determined analytical figures of merit limit of quantitation (LOD), limit of detection(LOQ), linearity, time per analysis, cost, sample preparation etc., are documented.

**8. Evaluation of method development with actual samples:**

The sample solution should lead to unequivocal, absolute identification of the analyte peak of interest apart from all other matrix components.

**9. Determination of percent recovery of actual sample and demonstration of quantitative sample analysis:**

Percent recovery of spiked, authentic standard analyte into a sample matrix that is shown to contain no analyte is determined. Reproducibility of recovery (average +/- standard deviation) from sample to sample and whether recovery has been optimized has been shown. It is not necessary to obtain 100% recovery as long as the results are reproducible and known with a high degree of certainity. The validation of analytical method can be verified only by laboratory studies. Therefore documentation of the

successful completion of such studies is a basic requirement for determining whether a method suitable for its intended application.

## ANALYTICAL METHOD VALIDATION

### Validation[10, 11]

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications.

### Reasons for validation

There are two important reasons for validating assays in the pharmaceutical industries. The frst, and by for the most important, is that assay validations is an integral part of the quality control system. The second is that current good manufacturing practice regulation requires assay validation.

### Typical validation characteristics

- Accuracy
- Precision
- Range
- Specificity
- Linearity
- Detection Limit
- Quantification Limit
- Ruggedness
- Robustness

**Accuracy**

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or on an accepted reference value and the value found.

**Precision**

It expresses as degree of agreement among individual test results when procedure/method is applied to a homogeneous sample – usually expressed as SD/RSD. It is a measure of degree of repeatability or reproducibility under normal conditions. A more comprehensive definition proposed by the ICH divides precision into three types

1. Repeatability.
2. Intermediate precision.
3. Reproducibility.

**Range**

The range of a method can be defined as the upper and lower concentrations for which the analytical method has adequate accuracy, precision and linearity. The range of concentrations examined will depend on the type of method and its use.

**Specificity**

Ability of the method to measure accurately and specifically the analyte of interest in presence of matrix and other components likely to be present in the sample matrix and impurities, degradation products and other related substances. For this, one may compare the test results of analysis of samples containing other ingredients/ impurities / degradation products / related substances/placebo ingredients with those obtained from analysis of sample without these, i.e., the method must allow distinct analytical measurement of analyte of interest and exclusion of all other relevant interferences.

If the impurities/degradation products or potential contaminants are not available, one can apply a proposed method to the strained and stressed (heat, light, humidity) samples. Degree of agreement among results will explain specificity of the method.

If the impurities/degradation products are not available, one may carryout additional purity tests by chromatography-HPLC/HPTLC.

**Linearity**

The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity can be assessed by performing single measurements at several analyte concentrations. A linearity correlation coefficient above 0.999 is acceptable for most methods, especially for major components in assay methods. The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample.

**Detection limit**

The Detection Limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The detection limit (LOD) may be expressed as

$$\mathbf{LOD} = \frac{\mathbf{3.3\sigma}}{\mathbf{S}}$$

Where,

$\sigma$ = the standard deviation of the response.

S = the slope of the calibration curve (of the analyte).

**Quantification limit**

LOQ is defined as the lowest concentration of the substance (analyte) in a sample that can be estimated quantitatively with acceptable precision, accuracy and reliability by a given method under stated experimental conditions. Quantification Limit (LOQ) may be expressed as

$$\mathbf{LOQ} = \frac{\mathbf{10\sigma}}{\mathbf{S}}$$

Where,

$\sigma$ = the standard deviation of the response.

S = the slope of the calibration curve (of the analyte).

**Ruggedness**

It is the measure of the capacity of the analytical method to remain unaffected by small but deliberate variations in procedure. It provides an indication about variability of the method during normal laboratory conditions.

**Robustness**

The concept of robustness of an analytical procedure has been defined by the ICH as "a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters". The most important aspect of robustness is to develop methods that allow for expected variations in the separation parameters.

**System suitability**

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Before performing any validation experiment, you should establish that the HPLC and the procedure are capable of providing data of acceptable quality. These tests are to verify that the resolution and repeatability of the system are adequate for the analysis to be performed. It is based on the concept that equipment, electronics, analytical operations and sample constitute an integral system that can be evaluated as a whole.

**System suitability parameters and recommendations**

| S. No | Parameters | Recommendations |
|---|---|---|
| 1 | Theoretical plates (N) | >2000 |
| 2 | Tailing factor (T) | ≤ 2 |
| 3 | Resolution (Rs) | > 2 between peak of interest and the closest eluting potential interference |
| 4 | Repeatability | RSD ≤ 1% for N ≥5 is desirable |
| 5 | Capacity factor ($k^1$) | > 2.0 |
| 6 | Relative retention | Not essential as long as the resolution is stated |

**System suitability parameters**[12]

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

The parameters that are affected by the changes in chromatographic conditions are,

- Column capacity factor ($K_A$)
- Resolution ($R_s$)
- Selectivity ($\alpha$)
- Column efficiency (N) and
- Peak asymmetry factor ($A_s$)

**i) Column capacity factor ($K_A$)**

The retention of a drug with a given packing material and eluent can be expressed as retention time or retention volume, but both of these are dependent on flow rate,

column length and column diameter. The retention is best described as a column capacity ratio (K), which is independent of these factors. The column capacity ratio of a compound (A) is defined as

$$K_A = \frac{V_A - V_0}{V_0} = \frac{t_A - t_0}{t_0}$$

Where,

$V_A$ = Elution volume of A

$V_0$ = Elution volume of a non retained compound (void volume).

**ii) Resolution ($R_s$)**

The resolution, $R_s$ of two neighboring peaks is defined by the ratio of the distance between the two peak maxima. It is the difference between the retention times of two solutes divided by their average peak width. For baseline separation, the ideal value of $R_s$ is 2.0. It is calculated by using the formula,

$$R_f = \frac{Rt_2 - Rt_1}{0.5\,(W_1 + W_2)}$$

Where,

$Rt_1$ and $Rt_2$ are the retention times of components 1 and 2

$W_1$ and $W_2$ are peak widths of components 1 and 2.

**iii) Selectivity ($\alpha$)**

The selectivity (or separation factor), $\alpha$, is a measure of relative retention of two components in a mixture. The ideal value of selectivity is 2. It can be calculated by using the formula,

$$\alpha = \frac{V_2 - V_0}{V_1 - V_0}$$

Where, $V_0$ is the void volume of the column and $V_2$ and $V_1$ are the retention volumes of the second and the first peak, respectively.

**iv) Column efficiency**

Efficiency, N, of a column is measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. Smaller the band spread, higher is the number of theoretical plates, indicating good column and system performance. Columns with N ranging from 5,000 to100,000 plates/meter are ideal for a good system. Efficiency is calculated by using the formula,

$$N = 16 \frac{Rt^2}{W^2}$$

Where, Rt is the retention time and W is the peak width.

**v) Peak asymmetry factor ($A_s$)**

Peak asymmetry factor, $A_s$ can be used as a criterion of column performance. The peak half width b of a peak at 10 % of the peak height, divided by the corresponding front half width a gives the asymmetry factor.

**vi) Tailing factor (T)**

A measure of the symmetry of a peak.

$$T = W_{0.05} / 2f$$

Where,

$W_{0.05}$ -peak width at 5% height

f -distance from peak front to apex point at 5% height.

The accuracy of quantification decreases with increase in peak tailing because of the difficulties encountered by the integrator in determining where/when the peak ends and hence the calculation of the area under the peak.

Limits-T </= 2

## STATISTICAL PARAMETERS[13, 14]

### Regression equations

The linear relationship is characterized by attendancy of the points of the scattered diagram to cluster along a straight line, known as the regression line.

$$\mathbf{Y= a + bX}$$

It is used to describe the dependence of one characteristic (Y) up on the other characteristic (X), both X,Y represent values of two characters, a and b are two constants it will be evident that two regression lines can be computed for every set of data-one each to describe the dependence of one character to another. b is known as regressive coefficients which show change expected in Y for unit change in X, it is dependence of Y & X; b is the regressive coefficient of Y& X.

The regressive coefficient of b is estimated,

$$b = \frac{\Sigma\left(x - \bar{x}\right)\left(y - \bar{y}\right)}{\Sigma\left(x - \bar{x}\right)^2}$$

b = the slope of the regression line and is calculated by this formula

x = an arbitrarily chosen value of the predictor variable for which the corresponding value of the criterion variable is desired.

### Correlation coefficient

A measure of the strength of the relationship between two variables is provided by the coefficient of correlation, denoted by r, if the relationship between the two variables is of the linear form. It is also called the coefficient of linear correlation.

### Standard deviation

It is the square root of the average of the squared deviations of the observations. From the arithmetic mean, it is used for measures of dispersion.

It is denoted by

**Standard Deviation** = $\sqrt{\frac{\Sigma(x-x)^2}{n-1}}$

**R.S.D (%)** = $\frac{\text{S.D}}{\bar{x}} \times 100$

Where,

| | | |
|---|---|---|
| $\Sigma$ | = | Sum of observations |
| $\bar{x}$ | = | Mean or arithmetic average ($\Sigma x / n$) |
| x | = | Individual observed value |
| $x - \bar{x}$ | = | Deviation of a value from the mean |
| n | = | Number of observations |

# AIM AND SCOPE OF THE WORK

The pharmaceutical formulations have gained a lot of importance in today's world due to greater patient acceptability, increased potency, multiple action, fewer side effects and quick action. Simultaneous analysis procedures are now being used more frequently for analysis of drugs in pharmaceutical formulations due to their advantages i.e. less time consuming, economical, accurate and precise.

The aim is development and validation of Metformin Hydrochloride (250mg) and Repaglinide (1mg) using parameters such precision, accuracy, linearity, specificity, robustness, ruggedness. Standard analytical procedure for newer drugs of formulation may not be available in pharmacopoeias; hence it is essential to develop newer analytical methods which are simple, accurate, precise, specific, economic, linear and rapid.

From the literature review it was found that a very few analytical methods have been reported for the simultaneous estimation of metformin hydrochloride and repaglinide by RP-HPLC.

Therefore in the proposed project, a successful attempt has been made to develop simple, accurate, economic and rapid methods for the estimation of tablet formulation and to validate the methods, as a result three simple, economic, precise and accurate methods were developed and validated by Reverse Phase High performance Liquid Chromatography

The method has been validated as per the guidelines given by ICH requirements to assure that the method consistently meets the predetermined specifications and quality attributes.

# PLAN OF WORK

## Literature survey

Through survey available for metformin hydrochloride and repaglinide, regarding their physical and chemical properties, pharmacology, pharmacokinetics and reported analytical methods, forms the basis for the development of new RP-HPLC method for simultaneous analysis of these drugs were designed.

## Procurement of samples

Procurement of the drugs specimens draws utmost priority. Both the drugs obtained from Chandra laboratories as samples and characterized by their melting points.

## Development of sample

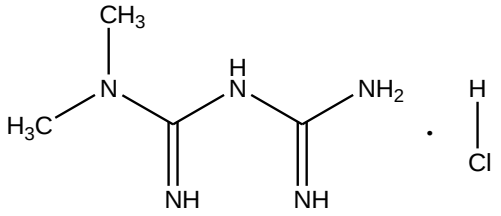
1. Selection of solvent system
2. Selection of mobile phase
3. Simultaneous method development for assay
4. Analysis of the commercially available formulations.

Analytical validations of development method according to ICH guideline parameters, which are selected for method validation, are as follows-

1. Precision
2. Linearity
3. Accuracy
4. Robustness
5. Ruggedness
6. System precision

## 1. METFORMINE HYDROCHLORIDE[15]

**Molecular structure**:




**Molecular weight** : 165.62

**Molecular formula** : $C_4H_{12}N_5Cl$

**Chemical name** : 1-carbamimidamido-N,N-dimethylmethanimidam HCl

**Category** : Hypoglycemic agent

**Description** : A white crystalline powder and highly hygroscopic

**Solubility** : Freely soluble in water, slightly soluble in alcohol, Practically insoluble in acetone, methylene chloride

**Melting point** : $222^0$ C-$226^0$ C

**Mechanism of action:**

Metformin's mechanisms of action differ from other classes of oral antihyperglycemic agents. Metformin decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization. These effects are mediated by the initial activation by metformin of AMP-activated protein kinase, a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats. Activation of AMP-activated protein kinase is required for metformin's inhibitory effect on the production of glucose by liver cells. Metformin administration also increases AMP-activated protein kinase

activity in skeletal muscle. AMP-activated protein kinase is known to cause GLUT4 deployment to the plasma membrane, resulting in insulin-independent glucose uptake.

**Pharmacodynamics:**

Metformin is an oral antihyperglycemic agent that improves glucose tolerance in patients with NIDDM, lowering both basal and postprandial plasma glucose. Metformin is not chemically or pharmacologically related to any other class of oral antihyperglycemic agents. Unlike sulfonylureas, metformin does not produce hypoglycemia in either patients with NIDDM or healthy subjects and does not cause hyperinsulinemia. Metformin does not affect insulin secretion.

**Pharmacokinetics:**

Metformin is metabolized. It is cleared from the body by tubular secretion and excreted unchanged in the urine, metformin id undetectable in blood plasma within 24 hrs of a single oral dose.

**Adverse effects:** Nausea, vomiting, anorexia, lactic acidosis, tolerance.

**Contraindications:** Diabetic ketoacidosis, cardiovascular collapse, renal failure, hepatic failure.

**Special precautions:** Alcohols not to be taken as an interaction occurs with metformin

**Half-life:** 6.2 hours

**Dosage**

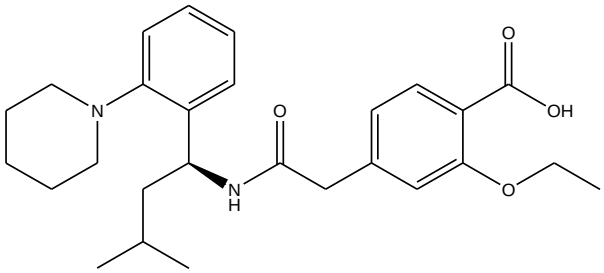
For adults, the initial dose is 250 mg twice or thrice a day with meals and increases gradually at 2 weeks intervals.

**Storage**

In a well closed container, stored at room temperature.

## 2. REPAGLINIDE[16]

**Molecular structure:**




**Molecular formula** : $C_{27}H_{36}N_2O_4$

**Molecular weight** : 452.58

**Chemical name** : 2-ethoxy-4-({[(1S)-3-methyl-1-[2-(piperidin-yl)phenyl]butyl]carbamoyl}methyl)benzoic acid

**Category** : Hypoglycemic acid

**Description** : A white crystalline powder and highly hygroscopic

**Solubility** : Freely soluble in methanol.

**Melting point** : 130-131$^0$ C

**Mechanism of action:**

Repaglinide activity is dependent on the presence functioning β cells and glucose. In contrast to sulfonylurea insulin secretatogogues, repaglinide has no effect on insulin release in the absence of glucose. Rather, it potentiates the effect of extracellular glucose on ATP-sensitive potassium channel and has little effect on insulin levels between meals and overnight. As such, repaglinide is more effective at reducing postprandial blood glucose levels than fasting blood glucose levels and requires a longer duration of therapy (approximately one month) before decreases in fasting

blood glucose are observed. The insulinotropic effects of repaglinide are highest at intermediate glucose levels (3 to 10 mmol/L) and it does not increase insulin release already stimulated by high glucose concentrations (greater than 15 mmol/L). Repaglinide appears to be selective for pancreatic β cells and does not appear to affect skeletal or cardiac muscle or thyroid tissue.

**Pharmacodynamic:**

Insulin secretion by pancreatic β cells is partly controlled by cellular membrane potential. Membrane potential is regulated through an inverse relationship between the activity of cell membrane ATP-sensitive potassium channels (ABCC8) and extracellular glucose concentrations. Extracellular glucose enters the cell via GLUT2 (SLC2A2) transporters. Once inside the cell, glucose is metabolized to produce ATP. High concentrations of ATP inhibit ATP-sensitive potassium channels causing membrane depolarization. When extracellular glucose concentrations are low, ATP-sensitive potassium channels open causing membrane repolarization. High glucose concentrations cause ATP-sensitive potassium channels to close resulting in membrane depolarization and opening of L-type calcium channels. The influx of calcium ions stimulates calcium-dependent exocytosis of insulin granules. Repaglinide increases insulin release by inhibiting ATP-sensitive potassium channels in a glucose-dependent manner.

**Pharmacokinetic:**

Absorption- Rapid and complete; peak plasma concentrations after 1 hour (oral).

Distribution-Protein-binding :> 98%.

Metabolism-Completely metabolized by oxidative biotransformation and direct

Conjugation with glucuronic acid.

Excretion-Urine (about 8%); feaces (90%)

**Adverse effects**:

Hypoglycaemia, nausea, diarrhoea, constipation, vomiting, dyspepsia, arthralgia, Sinusitis, rhinitis, back pain; rash, pruritus, urticaria; visual disturbances.

**Contraindications**

Diabetic ketoacidosis; severe hepatic impairment, type 1 diabetes; hypersensitivity. Lactation.

**Special precautions**

Myocardial infarction, coma, trauma during surgery, elderly, malnourished and debilitated patients. Hepatic or severe renal impairment. Pregnancy.

**Half-life:** 1 hour

**Dosage**

Adult: Usual initial dose: 0.5 mg, taken within 30 minutes of main meals. Initial doses of 1 or 2 mg may be used in patients who have had previous hypoglycemic treatment. May adjust dose at intervals of 1-2 weeks, up to 4 mg before meals.

**Storage:**

Store below 25°C. Protect from moisture.

## LITERATURE REVIEW

### Reported methods for Metformin hydrochloride

1) **Bhaskar Laxmanrao Kolte et al**[17] A simple, rapid, and precise reversed-phase liquid chromatographic method has been developed for the simultaneous determination of metformin in combination with glimepride. Under the developed conditions, good separation of the analytes was achieved in short analysis time. Several parameters affecting the separation of the analytes were studied, including pH and the concentration of SDS. The method is validated and shown to be linear in the range of 25 μg/mL to 150 μg/mL for metformin and 0.1 μg/mL to 0.6 μg/mL for glimepride. The method is applied for the analysis of these analytes in commercially available tablets.

2) **Aryane MS et al., 2006**[18] and coworkers carried out the development and validation of RP-HPLC method for the analysis of Metformin. In this a simple RP-HPLC method was developed for the quantification of Metformin hydrochloride in raw materials and in pharmaceutical preparations. Analytical Reverse Phase Column C(18) was used and the mobile phase consisted of methanol-water(30/70v/v) the analytes were then determined by using UV detector. This method was validated according to ICH guidelines. The proposed method is rapid, accurate, economical and selective and it was used for the quantitative analysis of metformin in Neodipar tablets because of its sensitivity and reproducibility.

3) **Sahoo P.K. et al., 2008**[19]**,** A high performance reverse phase liquid chromatographic procedure is developed for simultaneous estimation of metformin hydrochloride and pioglitazone hydrochloride in combined tablet dosage form. The mobile phase used was a combination of acetonitrile:water:acetic acid (60:40:0.3) and the pH was adjusted to 5.5 by adding triethylamine. The detection of the combined dosage form

was carried out at 230 nm and a flow rate employed was 1 ml/min. Linearity was obtained in the concentration range of 0.015 to 0.120 mg/ml of pioglitazone hydrochloride and 0.5 to 4.0 mg/ml of metformin hydrochloride with a correlation coefficient of 0.9992 and 0.9975. The results of the analysis were validated statistically and recovery studies confirmed the accuracy and precision of the proposed method.

4) **Sadaf Sayeed et al., 2009[20],** A simple, accurate and economic simultaneous equation method has been described for the simultaneous determination of metformin and pioglitazone in tablet dosage formulations. Metformin and pioglitazone showed absorption maxima at 233.5 nm and 266.5 nm respectively in 0.1 M NaOH prepared in glass double distilled water. The method allows rapid analysis of binary pharmaceutical formulations with high degree of accuracy and precision. Both the drugs showed linearity with absorbance in the concentration ranges, 5-40 mg / ml for Metformin and 10-80 mg / ml for pioglitazone. The results of the analysis have been validated statistically and by recovery studies. The method was also extending for dissolution studies.

5) **Mousumi kar et al., 2009**[21], simple, accurate, economical and reproducible HPLC method has been developed for quantitative estimation of metformin hydrochloride from tablet dosage form and formulated microspheres. The developed HPLC method is a reverse phase chromatographic method using phenomenex $C_{18}$ column and acetonitrile:phosphate buffer (65:35) pH adjusted to 5.75 with o-phosphoric acid as mobile phase and glipizide as internal standard. The linearity was observed in concentration range of 0-25 µg/ml for metformin hydrochloride. Results of analysis were validated statistically and by recovery studies.

6) **Lakshmi K.S. et al.,2009**[22], reported a simple, sensitive and rapid reverse phase high performance liquid chromatographic method was developed for the estimation of Metformin Hcl and Pioglitazone in pure and in pharmaceutical dosage forms. A Gemini C18 column (150x4.6mm, 5μ) was used with a mobile phase containing a mixture of Acetonitrile and Ammonium Acetate buffer (pH-3) in the ratio of 42: 58. The flow rate was 0.3ml/min and effluents were monitored at 255nm and eluted at 5.17min and 8.1min .Calibration curve was plotted with a range from 0.5-50 μg/ml for Metformin HCl and 0.3-30 μg/ml for Pioglitazone. The assay was validated for the parameters like accuracy, precision, robustness and system suitability parameters. The proposed method can be useful in the routine analysis for the determination of metformin and pioglitazone in pharmaceutical dosage forms.

7) **Onal A. et al, 2009**[23], carried out spectrophotometric and HPLC determinations of anti-diabetic drugs, Rosiglitazone maleate and Metformin hydrochloride, in pure form and in pharmaceutical preparations. In this method he developed three Spectrometric method and one HPLC method for analysis of Anti-diabetic drugs. Two Spectrometric methods were based on the reaction of rosiglitazone with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and bromocresol green, the third Spectrophotometric method consists of a zero-crossing first-derivative Spectrophotometric method for simultaneous analysis of RSG and metformin in tablets. The fourth method is a rapid stability indicating HPLC method. The proposed method was successfully applied to the tablet analysis.

8) **Narsimha rao Doredla et al, 2011**[24] developed a simultaneous estimation of Metformin HCl, pioglitazone HCl and glibenclamide in pure and tablet dosage form by using methanol as a solvent. Metformin HCl, pioglitazone HCl and glibenclamide show absorbance maxima at 237 nm, 270 nm and 230 nm respectively. Shimadzu UV

1700, capable of multicomponent analysis, was used for quantitation. This method is based on a multiwavelength spectroscopic method. Validation study reveals that the methods are specific, accurate, precise, and reproducible. All three drugs obey Beer's law in the concentration ranges used for the methods. Validation studies are statistically significant as all the statistical parameters are within the acceptance range for both accuracy and precision study. The methods are simple, rapid accurate, precise, reproducible, and economic and can be used for routine quantitative analysis of Metformin HCl, pioglitazone HCl and glibenclamide in pure and tablet dosage form.

9) ***Serasiya et al., 2011***[25], developed a simple, precise, specific and accurate reverse phase HPLC method has been for the simultaneous estimation of enalapril maleate (EM) and metformin hydrochloride (MT). The chromatographic separation was achieved on phenomenex Luna $C_{18}$ (25 cm Ã— 4.6 mm i.d., 5 Âµ) column using PDA detector. The mobile phase consisting of mixture of acetonitrile - 10mM $NaH_2PO_4$ (pH 2.2, adjusted with 80 % o-phosphoric acid) (30:70, v/v) at a flow rate of 1.0 ml/min was used. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness.

10) **Angshuman Biswas et al., 2011**[26]**,** A new simple, fast accurate and reproducible reverse phase high performance liquid chromatographic method has been developed and validated for simultaneous estimation of Metformin Hydrochloride and Glimepiride from tablet dosage form. The method was developed using Waters HPLC system on C18 column (Spherisorb ODS 2: 250mm x 4.6 µm) using a mixture of 25mM Phosphoric Acid pH 3.0 (with KOH) and Acetonitrile (40:60 v/v) as mobile phase in an isocratic elution mode at a flow rate of 1.00 ml/min at 40ºC with a load of 20µl. The detection was carried out using UV-Visible detector set at 240 nm. The

retention time of Metformin Hydrochloride and Glimepiride were found to be 3.20 min and 6.7 min respectively. The method was validated with respect to linearity, robustness, precision and accuracy. The method had been successfully applied in other pharmaceutical formulations of the same composition.

11) **Bhaskar Reddy et al., 2011**[27], Novel RP-HPLC method has been developed for simultaneous determination of Meformin HCl, Glipizide and Repaglinide in dosage forms. The separation was achieved on a 3.5-micron C18 column (150 X 4.6 mm) using mobile phase consisting of buffer (1.0gm of Potassium dihydrogen phosphate in 1000mL, pH 3.0 with diluted orthophospharic acid .The flow rate was maintained at 1.0 ml/min. The detection of the constituents was done using UV detector at 210 nm. The retention time of metformin, glipizide and repaglinide were approximately 1.49, 3.71 and 9.84 min respectively. Recovery study values of three actives were 103% to 99% respectively, relative standard deviation of less than 2%. Linear response obtained for three actives correlation coefficient is not less than 0.999. The proposed method was applied for

regular analysis and results found to be satisfactory.

12) **Bhamare P.C. et al., 2011**[28], reported a selective, precise, isocratic and accurate stability indicating reverse phase high performance liquid chromatography method have been developed for the simultaneous determination of Metformin hydrochloride and Fenofibrate present in multicomponent dosage forms. The HPLC method was carried out on Inertsil octadecylsilane $C_{18}$ (250 mm x 4.6 mm i.d., 5 μm particle size) column. A mobile phase composed of acetonitrile - water (adjusted to pH 3 using orthophosphoric acid) in proportion of 70:30 v/v, at flow rate of 1 ml/min was used for the separation. Detection was carried out at 250nm. Method was validated statistically and recovery studies were carried out. The proposed method has been

applied successfully to the analysis of cited drugs either in pure form or in pharmaceutical formulations with good accuracy and precision. The method herein described can be employed for quality control and routine analysis of drugs in pharmaceutical formulations.

**Reported methods for repaglinide**

1) **Venkatesh P. et al., 2006**[29], this paper describes a convenient method for the separation and simultaneous determination of six anti-diabetic drugs viz., glibenclamide, gliclazide, glipizide, pioglitazone, repaglinide and rosiglitazone in pharmaceutical formulations. Also, the assay has been shown applied to support quantification of the six anti-diabetic drugs in human plasma. The analytes were either injected directly onto the column after suitable dilution (pharmaceutical formulation analysis) or a simple extraction procedure, using acetonitrile, from human plasma spiked with anti-diabetic drugs and internal standard (IS). Ternary gradient elution at a flow rate of 1 mL/min was employed on an Intertisl ODS 3V column (4.6 x 250 mm, 5 microm) at ambient temperature. The mobile phase consisted of 0.01 m formic acid (pH 3.0), acetonitrile, Milli Q water and methanol. Celecoxib was used as an IS. The six anti-diabetic drugs were monitored at a wavelength of 260 nm. The nominal retention times of glibenclamide, gliclazide, glipizide, pioglitazone, repaglinide and rosiglitazone were 11.4, 13.3, 14.8, 17.6, 20.78, 22.1 and 25.4 min, respectively. The assay developed for formulation analysis was found to be accurate and precise. The calibration curves ranged from 0.1 to 100 microg/mL for all analytes with the exception of GLB, where the range was 0.3-100 microg/mL. The plasma assay was validated for parameters such as specificity, accuracy and extraction recovery. The proposed method is simple, selective and can be extended for routine analysis of anti-diabetics in pharmaceutical preparations and in biological matrices.

2) **Kaushal N. et al., 2010**[30], Spectrofluorimetric and high-performance liquid chromatography methods for estimation of repaglinide were developed. These methods were validated for estimation of repaglinide in tablets as well as in receptor fluid obtained during *in vitro* permeation studies. Repaglinide was observed to exhibit emission and excitation wavelengths, respectively, at 379 nm and 282 nm with linearity in the concentration range of 5-80 µg/ml. High-performance liquid chromatography analysis of repaglinide yielded retention time of 6.14 min with linearity ranging from 0.1-1.2 µg/ml concentration. Spectrofluorimetric analysis of repaglinide in tablets yielded results comparable to high performance liquid chromatography.

3) **Love kumar soni et al., 2012**[31]**,** A simple, rapid and specific reversed-phase high performance liquid chromatographic method for simultaneous analysis of metformin hydrochloride, and repaglinide in a tablet dosage form has been developed and validated. HPLC analysis was performed on a $C_{18}$ column with 90:10 (*v/v*) acetonitrile – water as mobile phase at a flow rate of 1.0 mL $min^{-1}$. UV detection was performed at 223 nm. Total run time was 10 min; metformin hydrochloride and repaglinide were eluted with retention times of 2.72 min and 6.13min, respectively. The method was validated for accuracy, precision, linearity, specificity, and sensitivity in accordance with ICH guidelines. Validation revealed that the method is specific, rapid, accurate, precise, reliable, and reproducible. The high recovery and low coefficients of variation confirmed the suitability of the method for simultaneous analysis of the two drugs in tablet dosage form.

4) **Deepa R. et al**[32]**.,** A simple, specific and accurate stability-indicating reversed phase high performance liquid chromatographic method was developed for the simultaneous determination of Repaglinide and Metformin hydrochloride. An isocratic RPHPLC

was achieved on younglin HPLC system using Varian C18 (250 -4.6 mm i.d, 5 m particle size) columnwith the mobile phase containing mixture of acetonitrile: 10mM ammonium acetate(pH 3.0, adjusted with phosphoric acid) (70 : 30, v/v). The flow rate was 1.0ml/min and the eluent was monitored at 230nm. The retention times of Repaglinide and Metformin hydrochloride were found to be 3.1 min and 5.58 min, respectively. Linearity was established for Repaglinide and Metformin hydrochloride in the range of 0.5-3 µg/ml and 200-1200 µg/ml, respectively. The percentage recoveries of Repaglinide and Metformin hydrochloride were found to be in the range of 99.87%±0.7 and 99.89%±0.15 respectively. Both the drugs were subjected to acid, alkali, oxidation, and dry heat degradation. The degradation studies indicated, Repaglinide and Metformin hydrochloride showed degradation in acid, alkaline, $H_2O_2$, and in dry heat condition. The degradation products of Repaglinide and Metformin hydrochloride were well resolved from the pure drug with significant differences in their retention time values. This method can be successfully employed for simultaneous quantitative analysis of Repaglinide and Metformin hydrochloride in bulk drugs and formulations.

## 4.1 MATERIALS

### Drug sample

Metformin hydrochloride and repaglinide sample obtained from Chandra labs Pvt., Ltd., Hyderabad.

### Formulation used

Prandimet tablets containing 500 mg metformin hydrochloride and 2 mg repaglinide were proceed from Chandra labs Pvt., Ltd., Hyderabad.

### Equipement used:

| S.No | Name | Model | Manufacturer/Supplier |
|---|---|---|---|
| 1. | Analytical balance | Unibloc | Shimadzu, Libror |
| 2. | pH meter | Eutech | Shimadzu. |
| 3. | HPLC | LC-2010 | Shimadzu Corporation, Japan |
| 4. | UV | UV-2550 | Shimadzu Corporation |

### Chemicals used:

| S.No | Chemicals | Grade | Manufacturer/Supplier |
|---|---|---|---|
| 1. | Water | HPLC | Microlabs |
| 2. | Methanol | HPLC | Merck |
| 3. | Acetonitrile | HPLC | Merck |
| 4. | Potassium dihydrogen phosphate | AR | Merck |
| 5. | Sodium dihydrogen phosphate | AR | Merck |
| 6. | 0.1M Sodium hydroxide | AR | Merck |
| 7. | Hydrochloric acid | AR | Merck |
| 8. | Triethylamine | AR | Spectrochem |
| 9. | Ortho phosphoric acid | AR | Merck |

## METHOD DEVELOPMENT

A method was developed for the determination of repaglinide and metformin HCl on HPLC by selecting the solubility, λmax and optimum mobile phase which gives good resolution of repaglinide and metformin HCl.

### SOLUBILITY

Solubility of drugs was observed by dissolving it in different solvents and it was found that drugs having good solubility in following solvents.

**Table 1: Solubility of drugs in different solvents**

| S.no | Solvent | Solubility | |
|---|---|---|---|
| | | Metformin Hydrochloride | Repaglinide |
| 1. | Water | + | + |
| 2. | Acetonitrile | + | + |
| 3. | 0.1N NaOH | + | + |
| 4. | 0.1N HCl | + | + |
| 5. | Methanol | + | + |

### SELECTION OF WAVELENGTH (λmax)

### Standard stock solution of repaglinide

Accurately 10 mg of repaglinide was weighed into a clean and dry 10 ml volumetric flask, dissolved with sufficient volume of mobile phase and then made up to the volume with mobile phase.

### Working standard solution

0.1 ml of the stock solution was further diluted in a 10 ml volumetric flask with mobile phase to get a concentration of 10 µg/ml.

**Standard stock solution of Metformin Hydrochloride**

Accurately 10 mg of Metformin HCl was weighed into a clean and dry 10 ml volumetric flask, dissolved with sufficient volume of mobile phase and then made up to the volume with mobile phase.

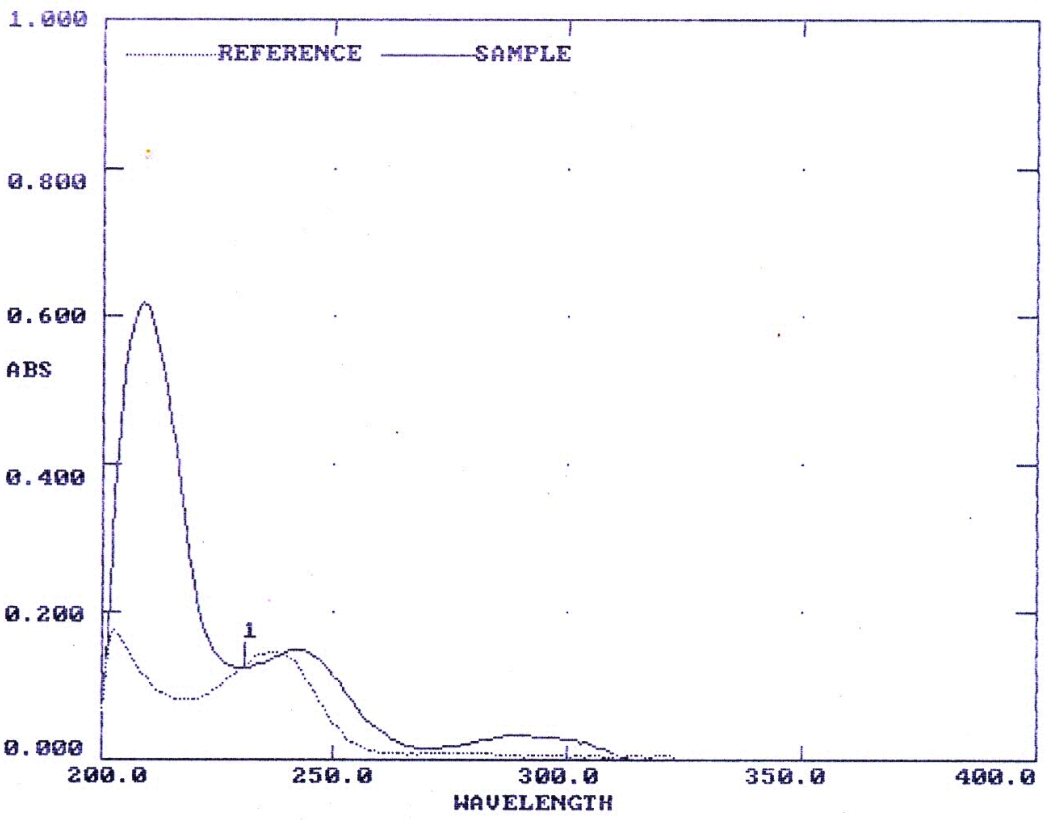
**Working standard solution**

0.1 ml of the stock solution was further diluted in a 10 ml volumetric flask with mobile phase to get a concentration of 10 µg/ml.

The overlaid UV spectrum of Metformin and Repaglinide was represented in fig-2.

**FIGURE-2**

**OVERLAID UV SPECTRUM OF REPAGLINIDE AND METFORMIN HYDROCHLORIDE**



## INITIALIZATION OF THE INSTRUMENT

Initially, the column was placed on the instrument and switch on the instruments and washed with methanol: water (20:80) for 30 min. then the system was made to run with the mobile phase for 30 min for column saturation.

### Standard preparation of Metformin HCl and Repaglinide

Standard-A: Accurately weighed quantity of 500 mg metformin HCl was transferred in to a 100 ml volumetric flask and made up to the volume with diluents. From this 5 ml was pipetted out in to a 50 ml volumetric flask and made up to the volume with same diluents.

Standard-B: Accurately weighed quantity of 2 mg repaglinide was transferred in to a 100 ml volumetric flask and made up to the volume with diluents. From this 5 ml was pipetted out in to a 50 ml volumetric flask and made up to the volume with same diluents.

**TRAIL-1**

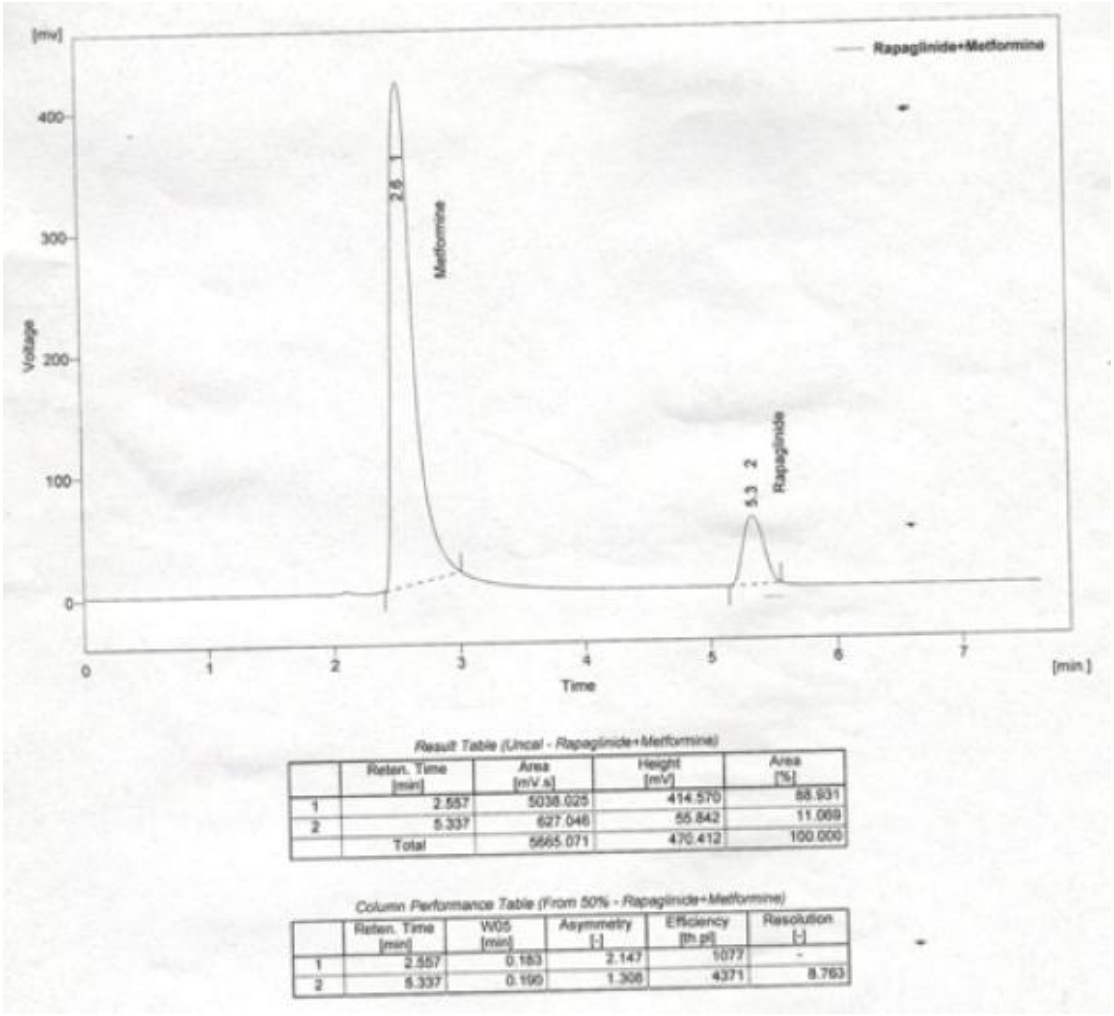
**1. Preparation of Buffer:**

0.01mole of potassium dihydrogen phosphate was dissolved in 1000ml of water and adjust the pH-3 using diluted O-phosphoric acid.

**2. Preparation of mobile phase:**

Filtered and degassed mixture of acetonitrile: buffer in the ratio of 80:20 and filter through 0.45 micron membrane filter (Fig-3).

**Fig: 3 Metformin HCl and Repaglinide**




*Result Table (Uncal - Rapaglinide+Metformine)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.557 | 5038.025 | 414.570 | 88.931 |
| 2 | 5.337 | 627.046 | 55.842 | 11.069 |
| | Total | 5665.071 | 470.412 | 100.000 |

*Column Performance Table (From 50% - Rapaglinide+Metformine)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.557 | 0.183 | 2.147 | 1077 | - |
| 2 | 5.337 | 0.190 | 1.308 | 4371 | 8.763 |

**Observation**: Assymetry of metformin HCl was not good.

**TRAIL-2**

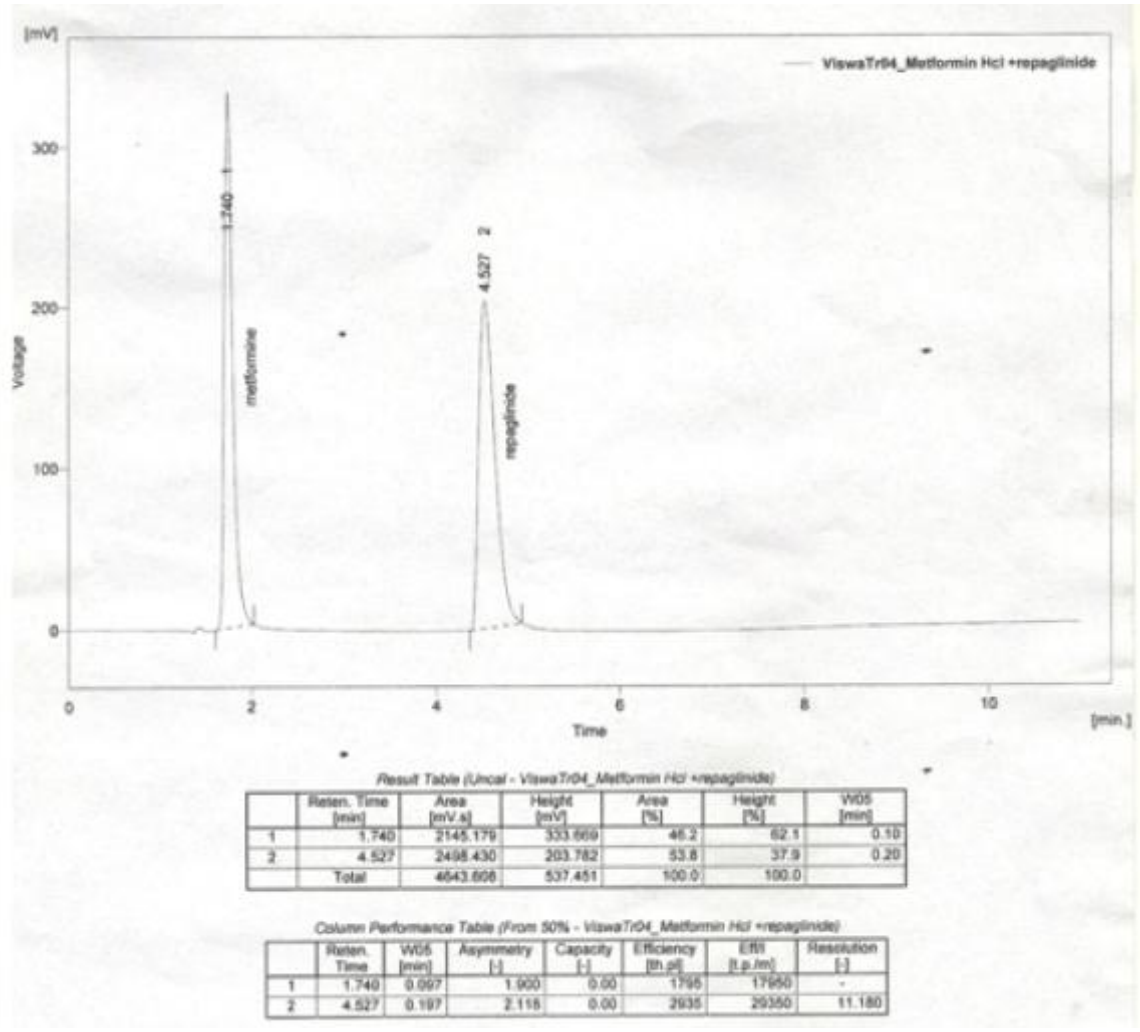
1. **Preparation of Buffer:**

0.01mole of potassium dihydrogen phosphate was dissolved in 1000ml of water and adjust the pH-3 using diluted O-phosphoric acid.

2. **Preparation of mobile phase:**

Filtered and degassed mixture of acetonitrile: buffer in the ratio of 60:40 and filter through 0.45 micron membrane filter (Fig- 4).

**Fig: 4 Metformin HCl and Repaglinide**




*Result Table (Uncal - ViswaTr04_Metformin Hcl +repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] | Height [%] | W05 [min] |
|---|---|---|---|---|---|---|
| 1 | 1.740 | 2145.179 | 333.669 | 46.2 | 62.1 | 0.10 |
| 2 | 4.527 | 2498.430 | 203.782 | 53.8 | 37.9 | 0.20 |
| | Total | 4643.608 | 537.451 | 100.0 | 100.0 | |

*Column Performance Table (From 50% - ViswaTr04_Metformin Hcl +repaglinide)*

| | Reten. Time | W05 [min] | Asymmetry [-] | Capacity [-] | Efficiency [th.pl] | Effi [t.p./m] | Resolution [-] |
|---|---|---|---|---|---|---|---|
| 1 | 1.740 | 0.097 | 1.900 | 0.00 | 1795 | 17650 | - |
| 2 | 4.527 | 0.197 | 2.116 | 0.00 | 2935 | 29350 | 11.180 |

**Observation**: Retention time of metformin HCl was not good.

**TRAIL-3**

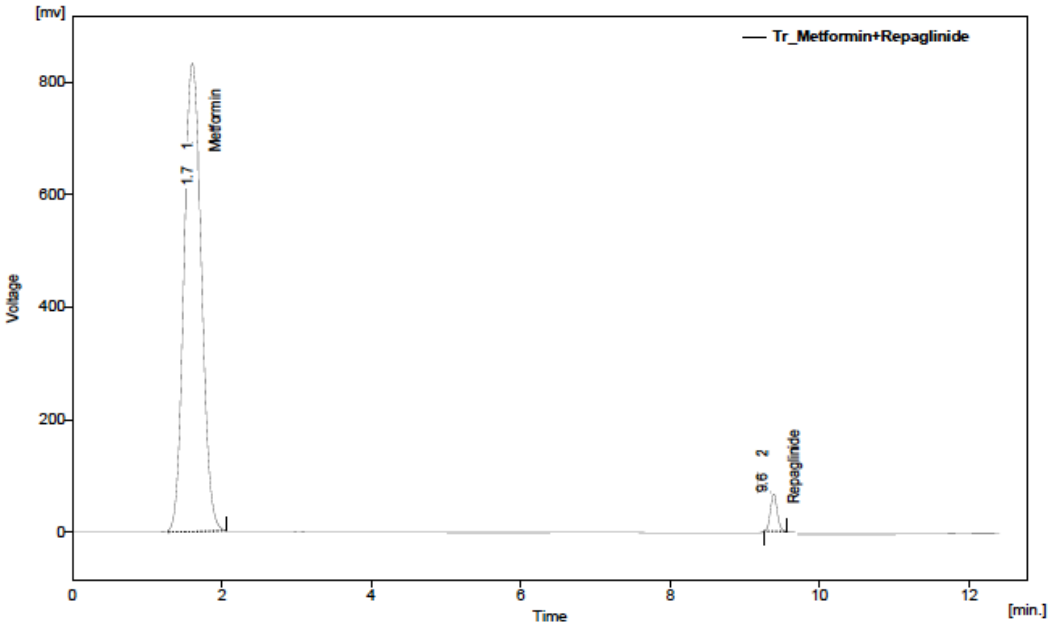
**1. Preparation of Buffer:**

0.01mole of potassium dihydrogen phosphate was dissolved in 1000ml of water and adjust the pH-3 using diluted O-phosphoric acid.

**2. Preparation of mobile phase:**

Filtered and degassed mixture of acetonitrile: buffer in the ratio of 50:50 and filter through 0.45 micron membrane filter (Fig-5).

**Fig: 5 Metformin HCl and Repaglinide**




*Result Table (Uncal - Tr_Metformin+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 1.673 | 418.712 | 67.051 | 3.015 |
| 2 | 9.627 | 13467.501 | 831.445 | 96.985 |
| | Total | 13886.213 | 898.497 | 100.000 |

*Column Performance Table (From 50% - Tr_Metformin+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 1.673 | 0.100 | 1.074 | 1551 | - |
| 2 | 9.627 | 0.257 | 1.159 | 7793 | 26.243 |

**Observation:**

Retention time of metformin HCl was not good.

**TRAIL-4**

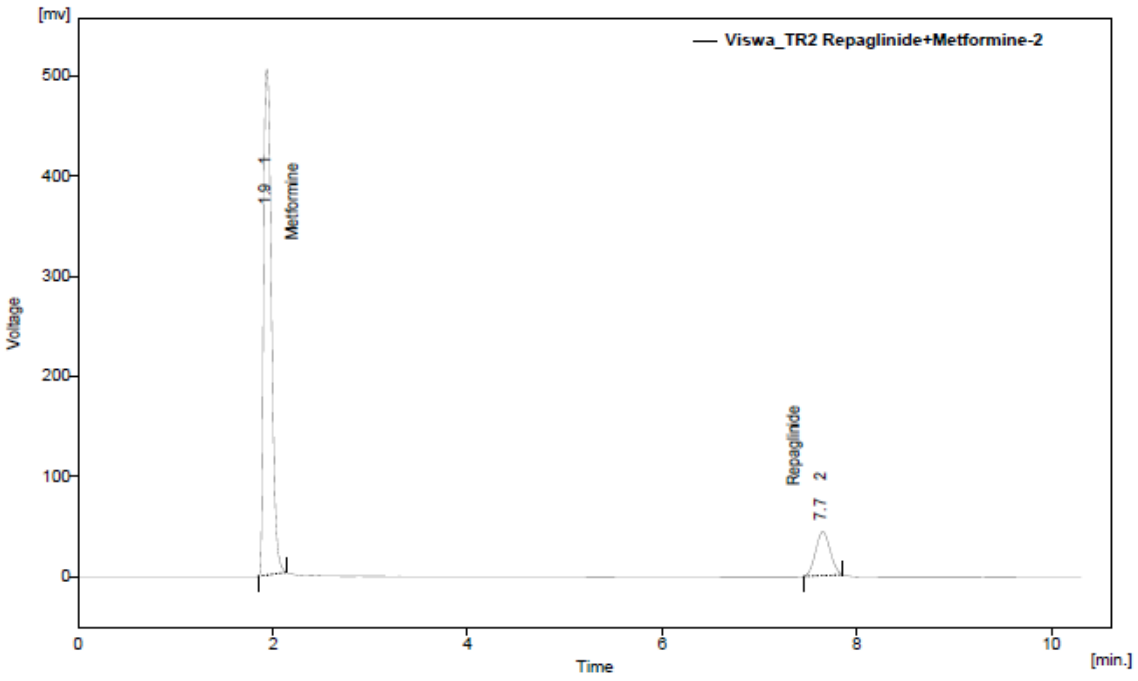
**1. Preparation of Buffer:**

0.01mole of sodium dihydrogen phosphate was dissolved in 1000ml of water and adjust the pH-3 using diluted O-phosphoric acid.

**2. Preparation of mobile phase:**

Filtered and degassed mixture of acetonitrile: buffer in the ratio of 60:40 and filter through 0.45 micron membrane filter (Fig-6).

**Fig: 6 Metformin HCl and Repaglinide**




*Result Table (Uncal - Viswa_TR2 Repaglinide+Metformine-2)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 1.940 | 2832.452 | 505.311 | 86.176 |
| 2 | 7.653 | 454.354 | 43.769 | 13.824 |
| | Total | 3286.806 | 549.080 | 100.000 |

*Column Performance Table (From 50% - Viswa_TR2 Repaglinide+Metformine-2)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 1.940 | 0.090 | 1.706 | 2574 | - |
| 2 | 7.653 | 0.167 | 1.093 | 11682 | 26.197 |

**Observation:** Retention time of metformin HCl was not good.

## OPTIMIZED METHOD

**1. Preparation of Buffer:**

0.01mole of sodium dihydrogen phosphate was dissolved in 1000ml of water and adjust the pH-3 using diluted O-phosphoric acid.

**2. Preparation of mobile phase:**

Filtered and degassed mixture of acetonitrile: buffer in the ratio of 58:42 and filter through 0.45 micron membrane filter.

**3. Preparation of standard stock solution**

An accurately weighed quantity of 500 mg of Metformin HCl and 2 mg of Repaglinide was transferred into 100 ml volumetric flask, dissolve in about 15 ml of mobile phase sonicate about 10 min until all the content has been dissolved, then the volume was made up to the mark with mobile phase. The concentrations of Metformin HCl and Repaglinide were found to be 5000 µg/ml and 20 µg/ml.

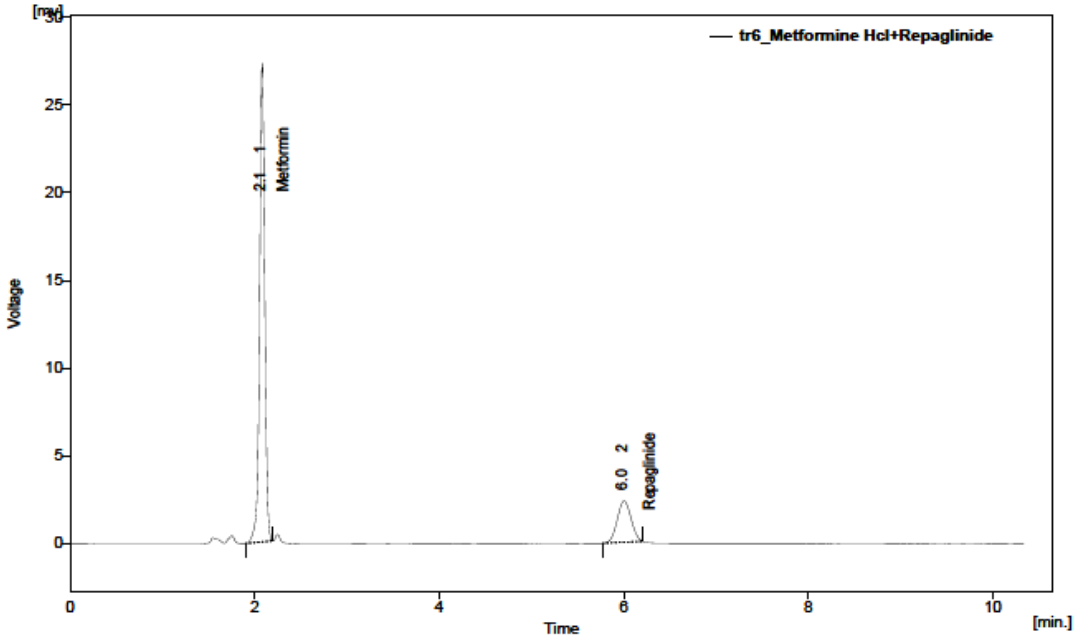
**4. Preparation of sample solution**

Weigh about 20 tablets and powdered. From that an equivalent amount of 500 mg of Metformin HCl and 1 mg of Propranolol hydrochloride was taken into 100 ml volumetric flask. Add about 10 ml of mobile phase and sonicate until the content was dissolved. Filter the content by using 0.45µ membrane filter by applying vacuum. Made the volume up to the mark with the mobile phase (Fig-7).

**Table 2: Optimized Chromatographic Condition**

| **Parameters** | **Description** |
|---|---|
| Mode of operation | Isocratic |
| Diluents | Water |
| Column | $C_{18}$, 250x4.6mm,5µ SS column |
| Mobile phase | Acetonitrile: buffer (58:42) |
| Flow rate | 1.0 ml/min |
| Detection of Metformin HCl and repaglinide | 230 nm |
| Temperature | $25^0$ C |
| Injection Volume | 20 µl |
| Run time | 20 min |
| Detector | UV detector |

**Fig: 7 Metformin HCl and Repaglinide**




*Result Table (Uncal - tr6_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.080 | 106.384 | 27.304 | 81.306 |
| 2 | 6.003 | 24.459 | 2.355 | 18.694 |
| | Total | 130.843 | 29.659 | 100.000 |

*Column Performance Table (From 50% - tr6_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.080 | 0.063 | 1.059 | 5975 | - |
| 2 | 6.003 | 0.167 | 1.093 | 7188 | 20.075 |

**Observation:** The retention time of Metformin HCl was 2.1 and Repaglinide was found to be 6.0 min. Theoretical plates, asymmetry are within the limits.

**Conclusion**

Out of the trials which were performed, the 5$^{th}$ trial was selected as the optimized condition for the method development, because the retention time was good, the asymmetry was found to be within the limits, the resolution was good.

## VALIDATION OF DEVELOPED RPHPLC METHOD FOR SIMULTANEOUS ESTIMATION OF REPAGLINIDE AND METFORMIN HCL

Since the HPLC method has been developed, validation of method using various parameters was performed to ensure that the performance characteristic of the method meets the requirements for the intended analytical applications.

### I.SYSTEM SUITABILITY

**System suitability studies**

From the standard solution of Repaglinide and Metformin hydrochloride five replicated injections were made. Various system suitability parameters like plate number (N), asymmetry factor, retention time, resolution, tailing factor, were evaluated from the standard chromatogram.

**Blank solution:** Purity water was used as diluents.

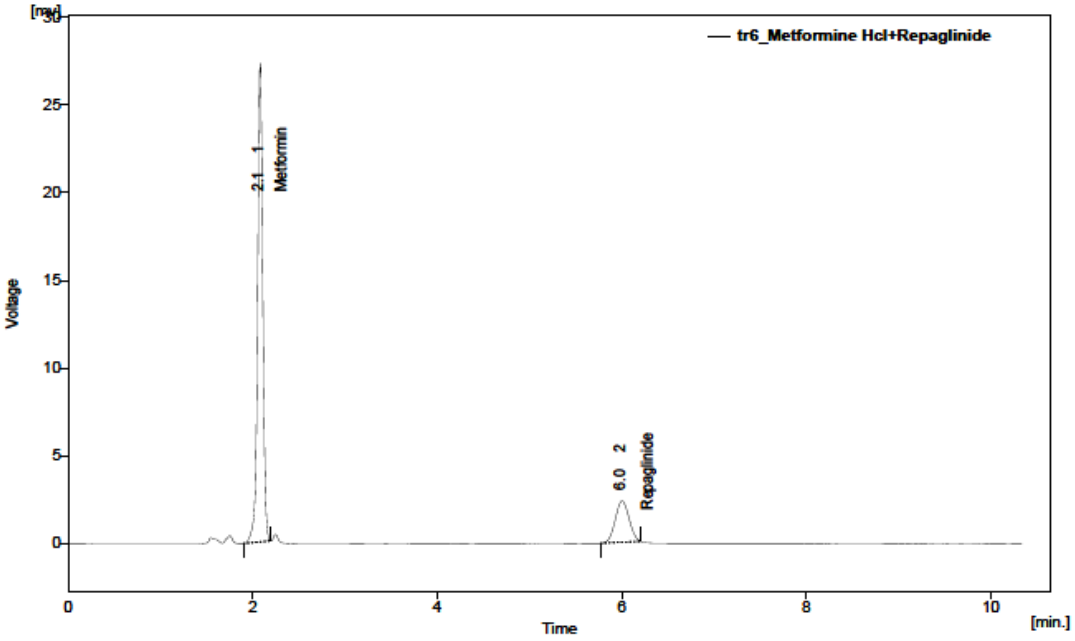
**Preparation of standard solution:**

An accurately weighed quantity of 500 mg metformin and 2 mg repaglinide was transferred into 100 ml volumetric flask, dissolved in about 15 ml of mobile phase and sonicated about 10 min until all the content has been dissolved, then the volume was made up to the mark with mobile phase.

**Preparation of test solution:**

About 0.819 g of the sample was taken in 100 ml volumetric flask and added 15 ml of mobile phase and sonicated for 15 min to dissolve the content and made upto the volume. From this 5 ml was pipette out into 100 ml volumetric flask and made upto the volume with same mobile phase. Filter the content by using 0.45µ membrane filter by applying vacuum (Fig-8). The results are tabulated in Table. No.-3

**Fig: 8 Metformin HCl and Repaglinide**




*Result Table (Uncal - tr6_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.080 | 106.384 | 27.304 | 81.306 |
| 2 | 6.003 | 24.459 | 2.355 | 18.694 |
| | Total | 130.843 | 29.659 | 100.000 |

*Column Performance Table (From 50% - tr6_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.080 | 0.063 | 1.059 | 5975 | - |
| 2 | 6.003 | 0.167 | 1.093 | 7188 | 20.075 |

## II. SPECIFICITY

For the simultaneous determination of Metformin HCl and Repaglinide, the specificity requires that the method should not be affected by the presence of other components. Usually the specificity would be performed by allowing the sample under stressed conditions.

### a) Treating with Acids

Take 1 ml from the stock solution into a 10 ml flask. To that flask add 1 ml of 0.1M hydrochloric acid. Observe for any change took place in the retention of the peak (Fig-9)

**b) Treating with Base**

Take 1 ml from stocks solution into a 10 ml flask, and add 1 ml of 0.1 M sodium hydroxide. Observe for any degradedness. (Fig-10)

**c) Heating**

For the specificity study 1 ml from the stock solution should be taken in a 10 ml flask, make up to the volume with the mobile phase. The solution should be heated at 40 $^{o}$ for a period of 30 min. Observe for any degradation occurs or not. (Fig-11)

**III. LINEARITY**

**Preparation of standard stock solution**

An accurately weighed quantity of 250 mg metformin and 1 mg repaglinide was transferred into 100 ml volumetric flask, dissolved in about 15 ml of mobile phase and sonicated about 10 min until all the content has been dissolved, then the volume was made up to the mark with mobile phase.

**Preparation of linearity solution-I:** Pipette out 1ml from stock solution to 100 ml with mobile phase. The concentration of the solution becomes 25µg of metformin and 0.1µg of repaglinide. (Fig-12)

**Preparation of linearity solution-II:** Pipette out 2ml from stock solution to 100 ml with mobile phase. The concentration of the solution becomes 50µg of metformin and 0.2µg of repaglinide. (Fig-13)

**Preparation of linearity solution-III:** Pipette out 3ml from stock solution to 100 ml with mobile phase. The concentration of the solution becomes 75µg of metformin and 0.3µg of repaglinide. (Fig-14)

**Preparation of linearity solution-IV:** Pipette out 4ml from stock solution to 100 ml with mobile phase. The concentration of the solution becomes 100µg of metformin and 0.4µg of repaglinide. (Fig-15)

**Preparation of linearity solution-V**: Pipette out 5ml from stock solution to 100 ml with mobile phase. The concentration of the solution becomes 125µg of metformin and 0.5µg of repaglinide. (Fig-16)

**Preparation of linearity solution-VI:** Pipette out 6ml from stock solution to 100 ml with mobile phase. The concentration of the solution becomes 150µg of metformin and 0.6µg of repaglinide. (Fig-17)

**Determination:** The linearity of the analytical method is determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range. Area is plotted graphically as a function of analyte concentration. Percentage curve fittings are calculated (Fig- 18).

The results are tabulated in Table. No- 4&5.

**IV. ACCURACY**

**Preparation of standard stock solution**

An accurately weighed quantity of 250 mg metformin and 1 mg repaglinide was transferred into 100 ml volumetric flask, dissolved in about 15 ml of mobile phase and sonicated about 10 min until all the content has been dissolved, then the volume was made up to the mark with mobile phase.

**Preparation of Spiking standard:** 5 ml from stock solution was further diluted into 100 ml with mobile phase (Fig- 19, 20, 21).

**Preparation of Accuracy solution 1:** Transfer 4ml from stock solution to 100 ml with mobile phase. The concentration of the solution becomes 112.5mcg of metformin and 0.4mcg of repaglinide and added 1 ml of spiking standard (Fig- 22, 23, 24).

**Preparation of Accuracy solution 2:** Pipette out 5ml from stock solution to 100 ml with mobile phase. The concentration of the solution becomes 137.5mcg of metformin and 0.5mcg of repaglinide and added 1 ml of spiking standard (Fig- 25, 26, 27).

**Preparation of Accuracy solution 3:** Pipette out 6ml from stock solution to 100 ml with mobile phase. The concentration of the solution becomes 162.5mcg of metformin and 0.6mcg of repaglinide and adds 1 ml of spiking standard (Fig- 28, 29, 30).

**Determination:** the accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay. The accuracy studies for Repaglinide and Metformin HCl was carried out at three different levels (Fig-31).

The results are tabulated in Table. No- 6&7.

## V. PRECISION

### A) Repeatability:

Established the repeatability of the analytical method by estimating the assay for 5 sample proportion of the same batch under normal operating conditions. Calculated the assay for all 5 sample preparation and reported the %RSD for the same (Fig-32 to 36).

**Standard preparation:**

An accurately weighed quantity of 250 mg metformin and 1 mg repaglinide was transferred into 100 ml volumetric flask, dissolved in about 15 ml of mobile phase and sonicated about 10 min until all the content has been dissolved, then the volume was made up to the mark with mobile phase.

**Preparation of test solution:**

About 0.819 g of the sample was taken in 100 ml volumetric flask and added 15 ml of mobile phase and sonicated for 15 min to dissolve the content and made upto the volume. From this 5 ml was pipette out into 100 ml volumetric flask and made up to the volume with same mobile phase. Filter the content by using 0.45µ membrane filter by applying vacuum.

The results are tabulated in Table. No-8.

### B) Intermediate precision (ruggedness)

Intermediate precision study was carried out by repeating the complete experiment with different analysts, on different days in same laboratory as per the following preparation.

**Standard preparation:**

**A**n accurately weighed quantity of 250 mg metformin and 1 mg repaglinide was transferred into 100 ml volumetric flask, dissolved in about 15 ml of mobile phase and sonicated about 10 min until all the content has been dissolved, then the volume was made up to the mark with mobile phase.

**Preparation of test solution:**

About 0.819 g of the sample was taken in 100 ml volumetric flask and added 15 ml of mobile phase and sonicated for 15 min to dissolve the content and made upto the volume. From this 5 ml was pipette out into 100 ml volumetric flask and made upto the volume with same mobile phase. Filter the content by using 0.45µ membrane filter by applying vacuum (Fig-37&38).

**Determination:** The precision of the analytical method and instrument is determined by assaying sufficient number of samples and relative standard deviation is calculated.

The results are tabulated in Table. No-9.

**C) Method precision**

**Standard preparation:**

An accurately weighed quantity of 250 mg metformin and 1 mg repaglinide was transferred into 100 ml volumetric flask, dissolved in about 15 ml of mobile phase and sonicated about 10 min until all the content has been dissolved, then the volume was made up to the mark with mobile phase.

**Preparation of test solution:**

About 0.819 g of the sample was taken in 100 ml volumetric flask and added 15 ml of mobile phase and sonicated for 15 min to dissolve the content and made upto the volume. From this 5 ml was pipette out into 100 ml volumetric flask and made upto

the volume with same mobile phase. Filter the content by using 0.45µ membrane filter by applying vacuum. (Fig-39 to 43)

The results are tabulated in Table. No-10.

**VI. ROBUSTNESS**

**Blank solution:** Purity water was used as diluents.

**Standard preparation:**

An accurately weighed quantity of 250 mg metformin and 1 mg repaglinide was transferred into 100 ml volumetric flask, dissolved in about 15 ml of mobile phase and sonicated about 10 min until all the content has been dissolved, then the volume was made up to the mark with mobile phase.

**Preparation of test solution:**

About 0.819 g of the sample was taken in 100 ml volumetric flask and added 15 ml of mobile phase and sonicated for 15 min to dissolve the content and made upto the volume. From this 5 ml was pipette out into 100 ml volumetric flask and made upto the volume with same mobile phase. Filter the content by using 0.45µ membrane filter by applying vacuum. (Fig-44-47)

| S. No | Chromatographic condition | Low | High |
|---|---|---|---|
| 1. | Flow rate | 0.9 ml | 1.1 ml |
| 2. | Wavelength | 228 nm | 232 nm |

**Determination:** the robustness of an analytical method is to determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay.

The results are tabulated in Table. No-11&12.

**ASSAY**

Weigh about 20 tablets and powdered. An equivalent weight of 500 mg and 2mg of Metformin HCl and Repaglinide were taken into 50 ml volumetric flask. Add about 10 ml of mobile phase and sonicate until the contents were dissolved. Filter the contents by using 0.45µ membrane filter under vaccum. Make up to the mark with mobile phase. Inject 20µl of sample solution into the chromatographic system. Measure the area of Metformin HCl and Repaglinide and calculate the percentage of assay.

Formula:

$$= \frac{\text{Avg sample area x Standard wt x Tablet wt taken x Standard purity}}{\text{Avg std area x Sample wt x 100}}$$

$$\text{Assay \%} = \frac{\text{Amount of sample}}{\text{Label claim}} \times 100$$

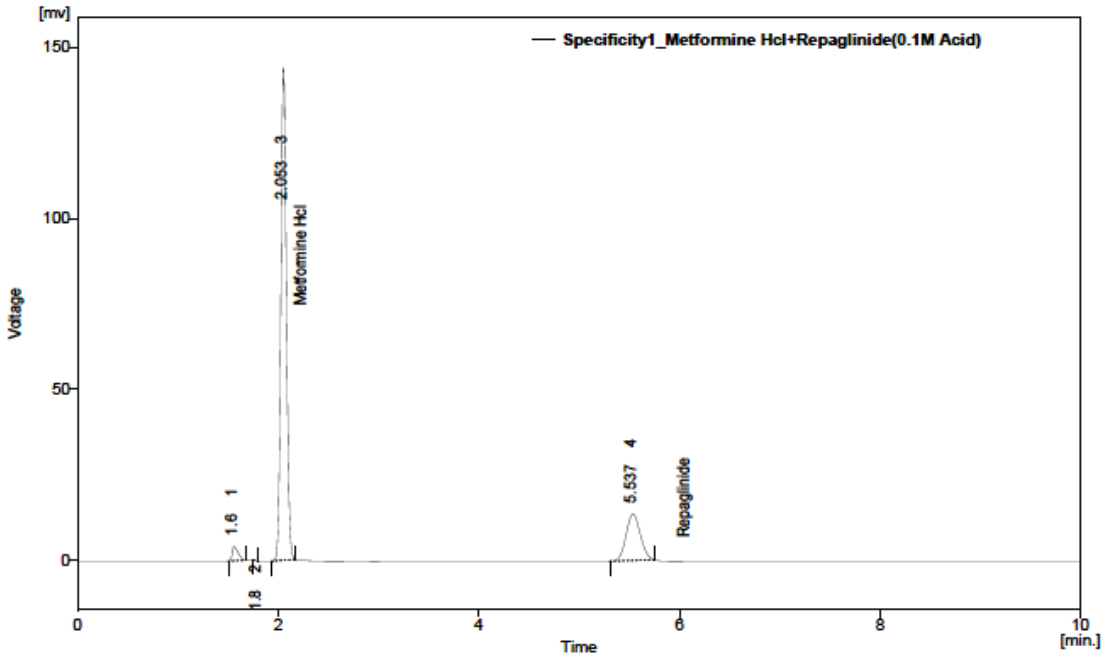
# CHROMATOGRAMS

## SPECIFICITY

**FIGURE – 9**

### SPECIFICITY CHROMATOGRAM– I

### METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg) 0.1 M ACID




*Result Table (Uncal - Specificity1_Metformine Hcl+Repaglinide(0.1M Acid))*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 1.560 | 17.691 | 4.321 | 2.549 |
| 2 | 1.797 | 0.112 | 0.032 | 0.016 |
| 3 | 2.053 | 542.348 | 144.048 | 78.134 |
| 4 | 5.537 | 133.975 | 13.658 | 19.301 |
| | Total | 694.127 | 162.059 | 100.000 |

*Column Performance Table (From 50% - Specificity1_Metformine Hcl+Repaglinide(0.1M Acid))*

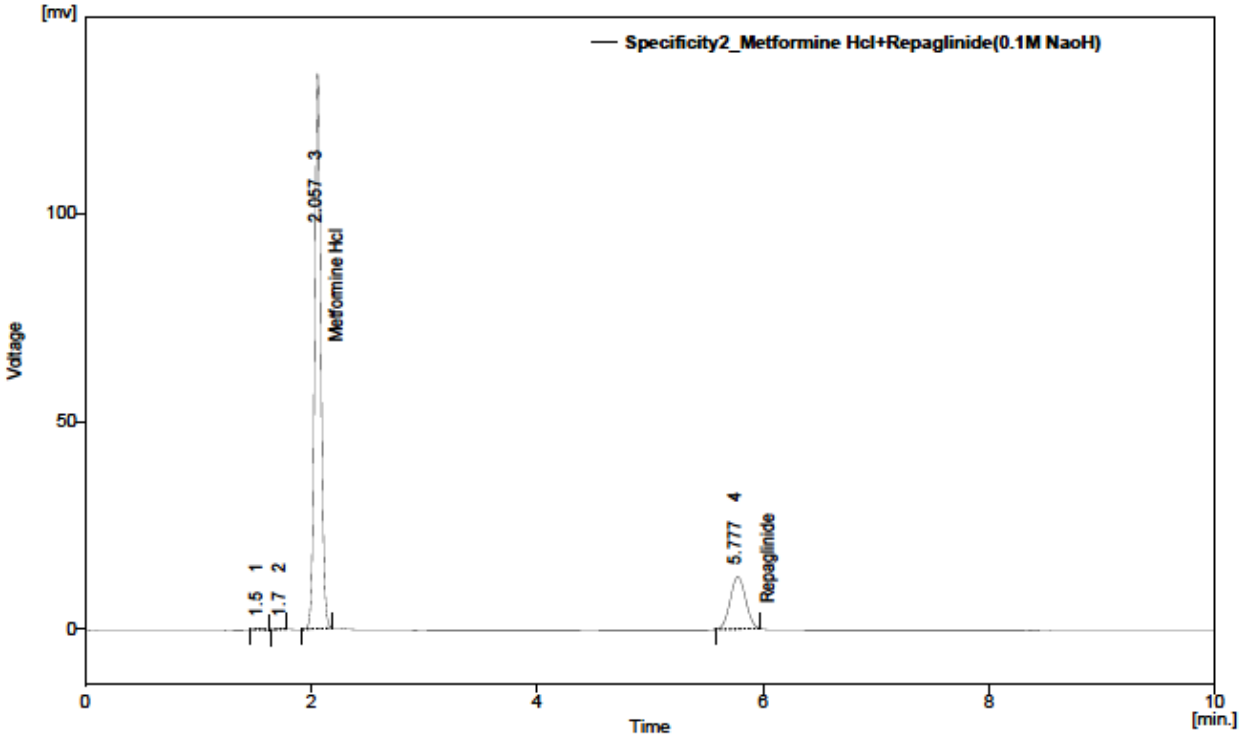
| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 1.560 | 0.067 | 2.700 | 3033 | - |
| 2 | 1.797 | 0.013 | 0.143 | 100593 | 3.482 |
| 3 | 2.053 | 0.060 | 1.125 | 6488 | 4.119 |
| 4 | 5.537 | 0.157 | 1.150 | 6919 | 18.920 |

**FIGURE – 10**

## SPECIFICITY CHROMATOGRAM– II

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)

## 0.1M NaOH




*Result Table (Uncal - Specificity2_Metformine Hcl+Repaglinide(0.1M NaoH))*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 1.537 | 1.752 | 0.437 | 0.276 |
| 2 | 1.737 | 1.523 | 0.342 | 0.240 |
| 3 | 2.057 | 511.244 | 133.422 | 80.549 |
| 4 | 5.777 | 120.179 | 12.616 | 18.935 |
| | Total | 634.698 | 146.817 | 100.000 |

*Column Performance Table (From 50% - Specificity2_Metformine Hcl+Repaglinide(0.1M NaoH))*

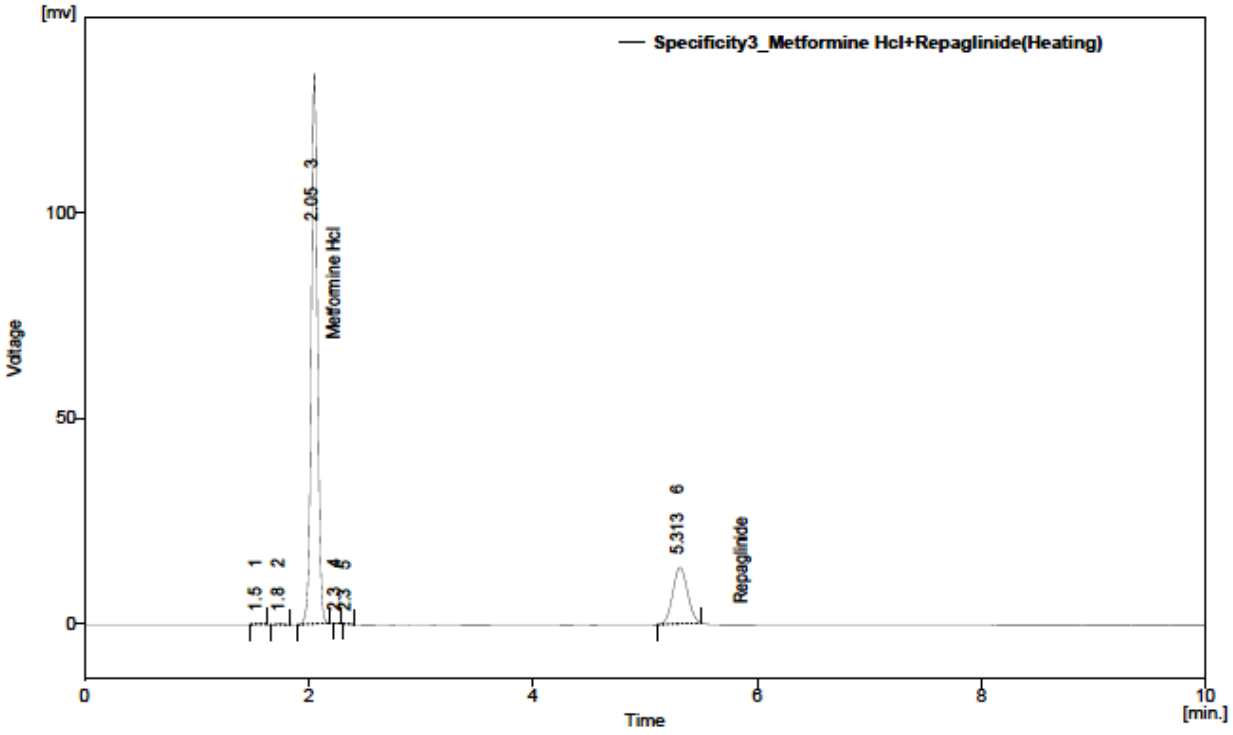
| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 1.537 | 0.060 | 1.600 | 3634 | - |
| 2 | 1.737 | 0.073 | 0.542 | 3107 | 1.765 |
| 3 | 2.057 | 0.063 | 1.188 | 5842 | 2.756 |
| 4 | 5.777 | 0.153 | 1.075 | 7863 | 20.206 |

**FIGURE – 11**

## SPECIFICITY CHROMATOGRAM– III

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)

## HEATING




*Result Table (Uncal - Specificity3_Metformine Hcl+Repaglinide(Heating))*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 1.547 | 1.571 | 0.405 | 0.246 |
| 2 | 1.750 | 1.686 | 0.388 | 0.264 |
| 3 | 2.050 | 509.810 | 133.986 | 79.797 |
| 4 | 2.250 | 0.497 | 0.205 | 0.078 |
| 5 | 2.340 | 0.235 | 0.070 | 0.037 |
| 6 | 5.313 | 125.084 | 13.741 | 19.579 |
| | Total | 638.884 | 148.795 | 100.000 |

*Column Performance Table (From 50% - Specificity3_Metformine Hcl+Repaglinide(Heating))*

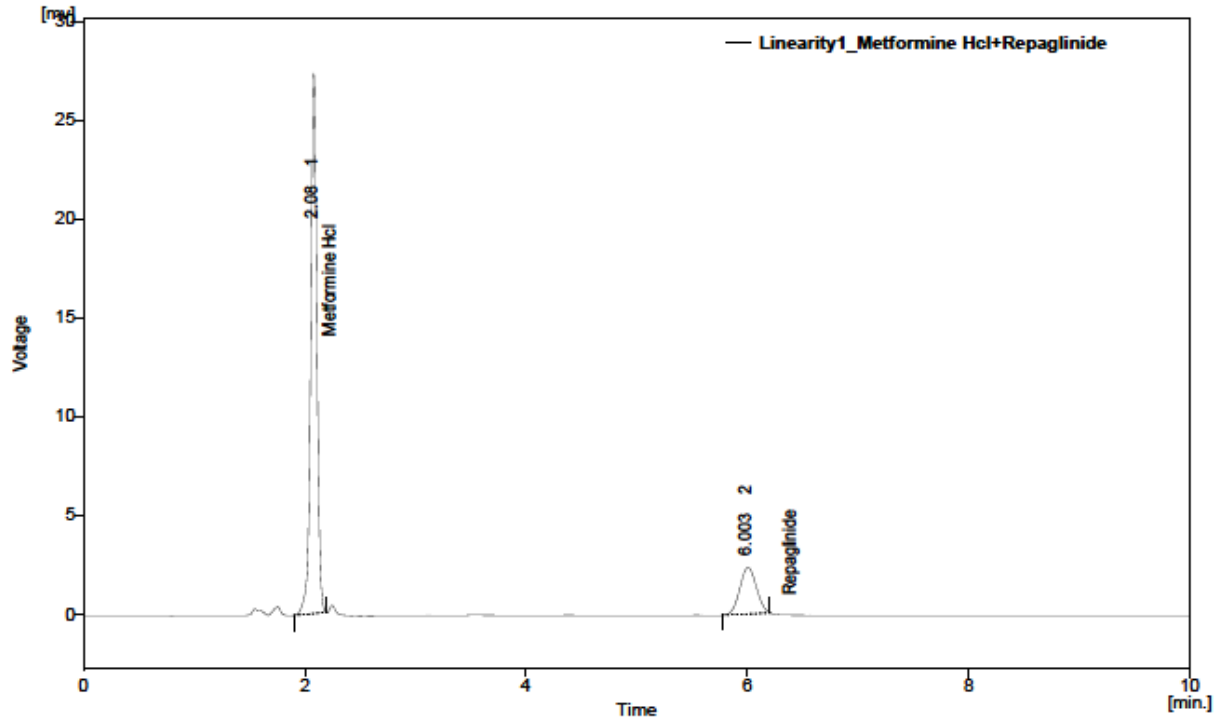
| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 1.547 | 0.063 | 1.846 | 3304 | - |
| 2 | 1.750 | 0.073 | 0.583 | 3155 | 1.751 |
| 3 | 2.050 | 0.060 | 1.125 | 6467 | 2.648 |
| 4 | 2.250 | 0.043 | 1.222 | 14936 | 2.278 |
| 5 | 2.340 | 0.053 | 1.889 | 10665 | 1.096 |
| 6 | 5.313 | 0.147 | 1.079 | 7271 | 17.496 |

## LINEARITY

**FIGURE – 12**

## LINEARITY CHROMATOGRAM– I

## METFORMIN HYDROCHLORIDE (25 µg) AND REPAGLINIDE (0.10 µg)




*Result Table (Uncal - Linearity1_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.080 | 102.384 | 27.304 | 81.306 |
| 2 | 6.003 | 24.459 | 2.355 | 18.694 |
| | Total | 126.843 | 29.659 | 100.000 |

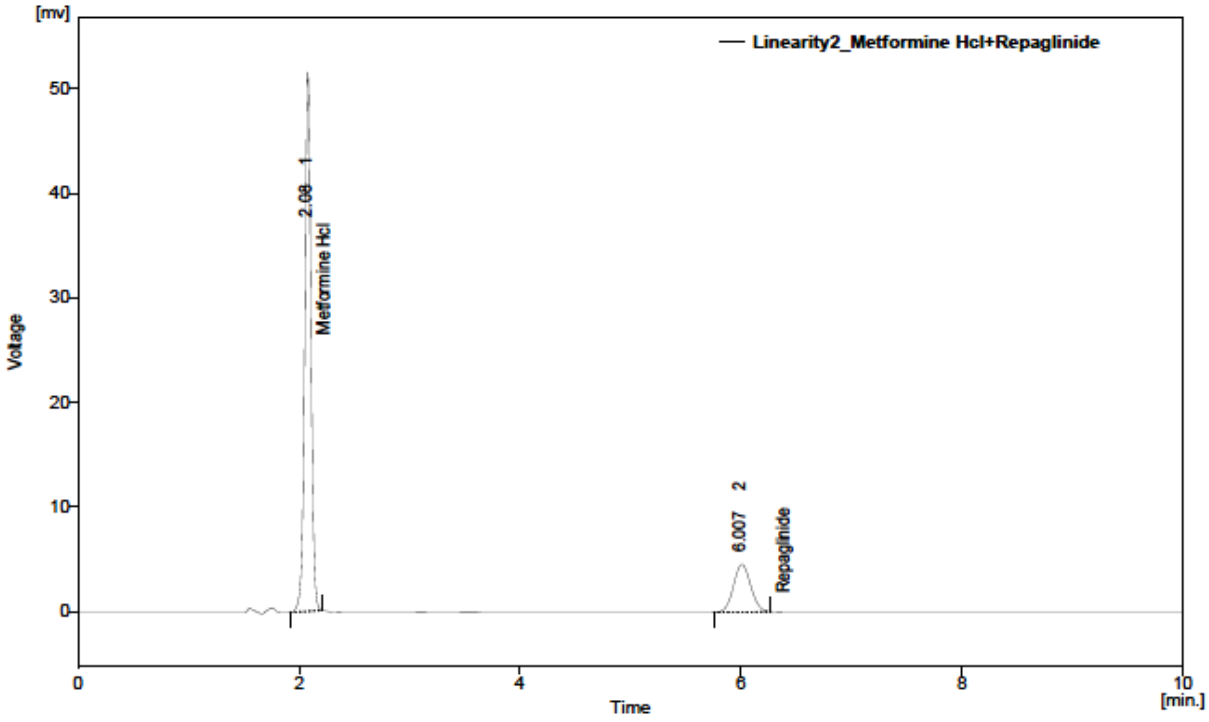
*Column Performance Table (From 50% - Linearity1_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.080 | 0.063 | 1.059 | 5975 | - |
| 2 | 6.003 | 0.167 | 1.093 | 7188 | 20.075 |

**FIGURE – 13**

## LINERARITY CHROMATOGRAM– II

## METFORMIN HYDROCHLORIDE (10 µg) AND REPAGLINIDE (0.04 µg)




*Result Table (Uncal - Linearity2_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.080 | 201.721 | 51.516 | 80.349 |
| 2 | 6.007 | 49.334 | 4.546 | 19.651 |
| | Total | 251.055 | 56.062 | 100.000 |

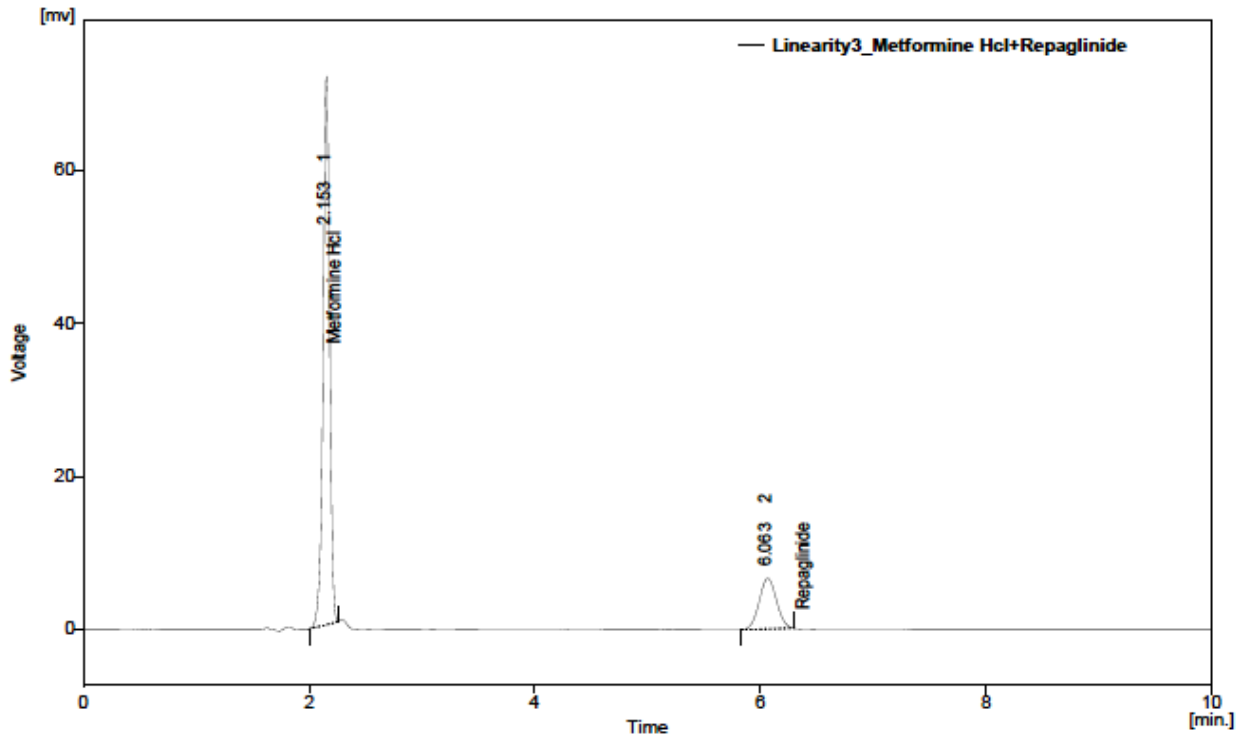
*Column Performance Table (From 50% - Linearity2_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.080 | 0.060 | 1.059 | 6658 | - |
| 2 | 6.007 | 0.173 | 1.111 | 6653 | 19.805 |

**FIGURE – 14**

## LINERARITY CHROMATOGRAM– III

## METFORMIN HYDROCHLORIDE (75 µg) AND REPAGLINIDE (0.3 µg)




*Result Table (Uncal - Linearity3_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.153 | 296.203 | 71.712 | 80.223 |
| 2 | 6.063 | 70.556 | 6.626 | 19.777 |
| | Total | 366.759 | 78.338 | 100.000 |

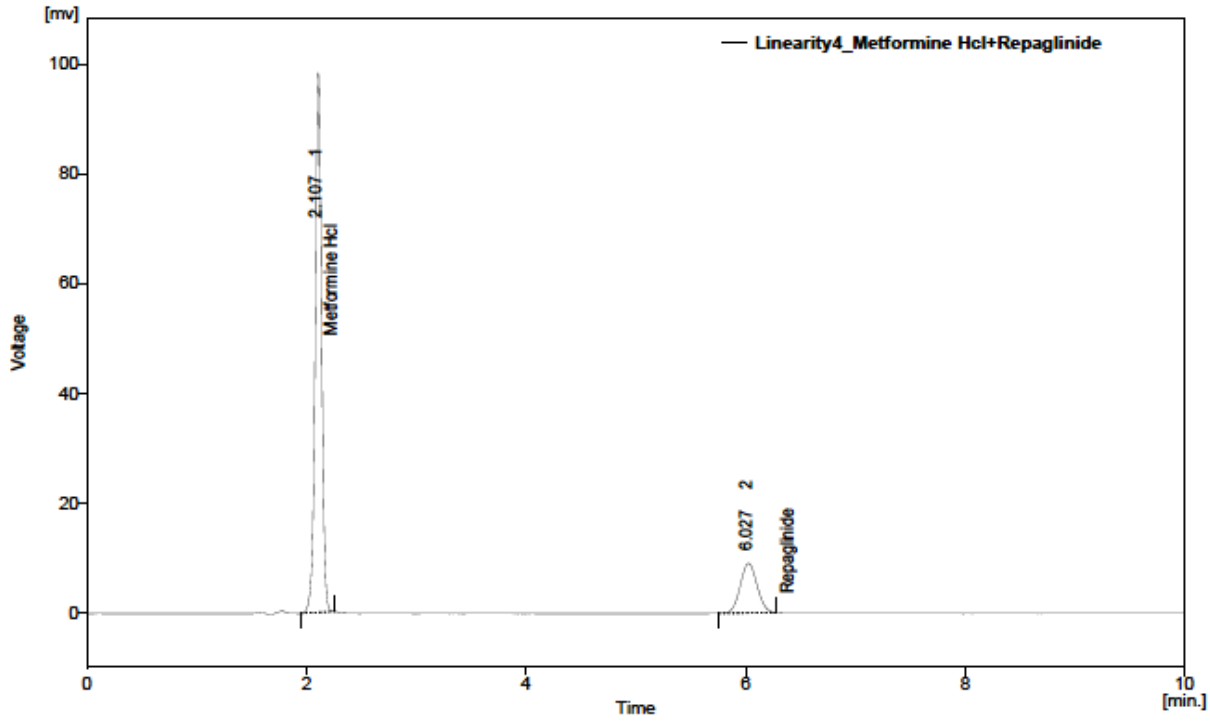
*Column Performance Table (From 50% - Linearity3_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.153 | 0.063 | 0.947 | 6404 | - |
| 2 | 6.063 | 0.173 | 1.091 | 6779 | 19.443 |

**FIGURE – 15**

## LINERARITY CHROMATOGRAM– IV

## METFORMIN HYDROCHLORIDE (100 µg) AND REPAGLINIDE (0.4 µg)




*Result Table (Uncal - Linearity4_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.107 | 393.970 | 98.307 | 80.202 |
| 2 | 6.027 | 97.251 | 9.089 | 19.798 |
| | Total | 491.221 | 107.396 | 100.000 |

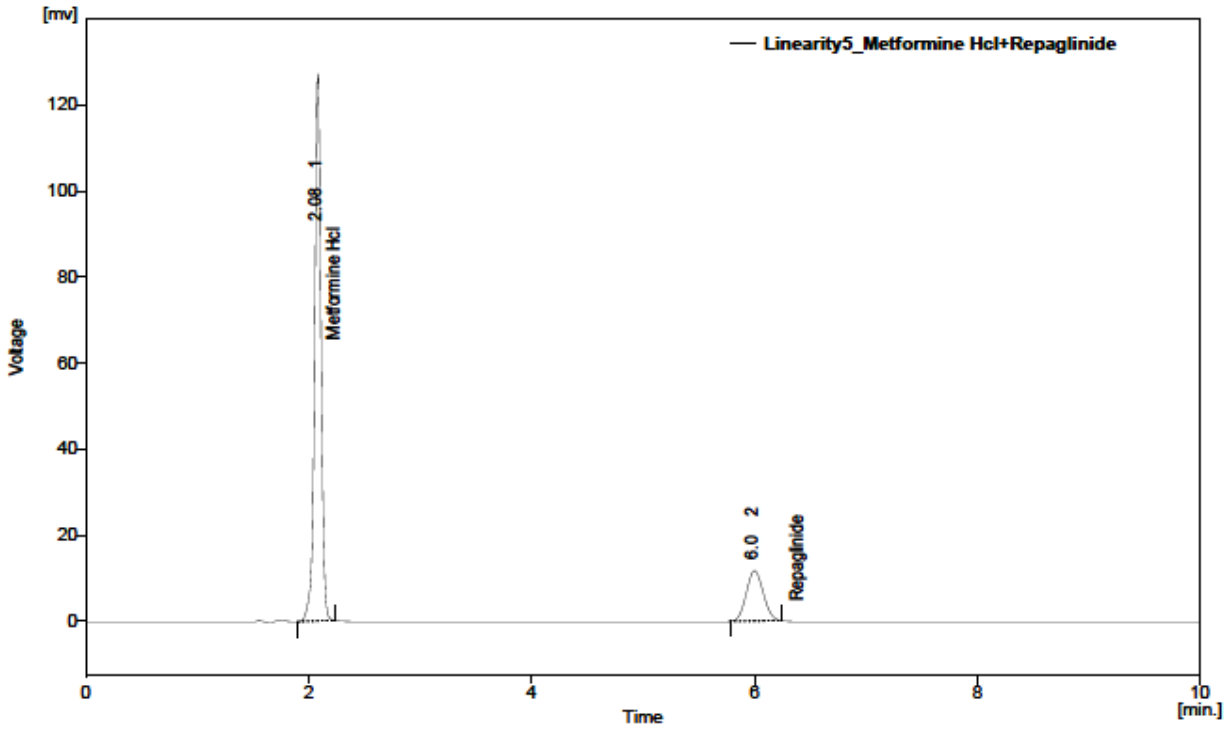
*Column Performance Table (From 50% - Linearity4_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.107 | 0.063 | 1.056 | 6130 | - |
| 2 | 6.027 | 0.170 | 1.114 | 6963 | 19.771 |

**FIGURE – 16**

## LINERARITY CHROMATOGRAM– V

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - Linearity5_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.080 | 505.630 | 127.101 | 80.320 |
| 2 | 6.000 | 123.888 | 11.716 | 19.680 |
| | Total | 629.518 | 138.817 | 100.000 |

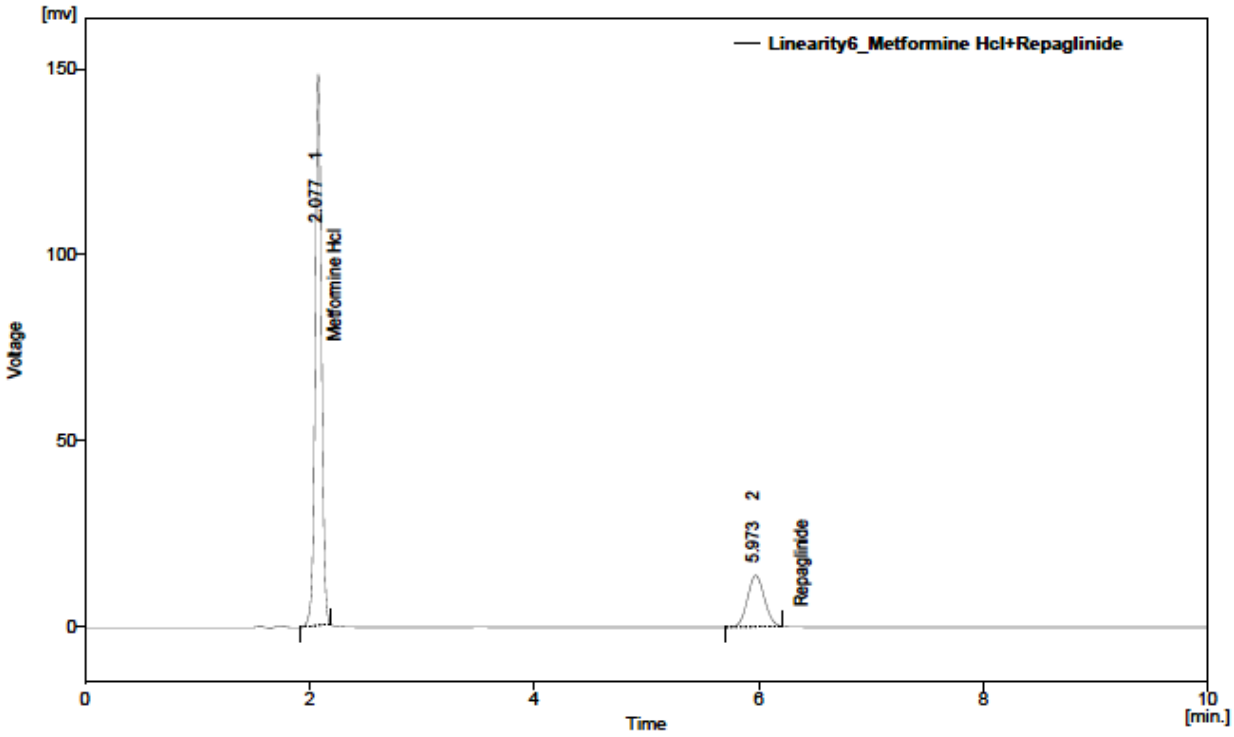
*Column Performance Table (From 50% - Linearity5_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.080 | 0.063 | 1.118 | 5975 | - |
| 2 | 6.000 | 0.167 | 1.140 | 7180 | 20.058 |

**FIGURE – 17**

## LINERARITY CHROMATOGRAM– VI

## METFORMIN HYDROCHLORIDE (150 µg) AND REPAGLINIDE (0.6 µg)




*Result Table (Uncal - Linearity6_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.077 | 599.490 | 148.061 | 79.959 |
| 2 | 5.973 | 147.491 | 13.774 | 20.041 |
| | Total | 746.981 | 161.835 | 100.000 |

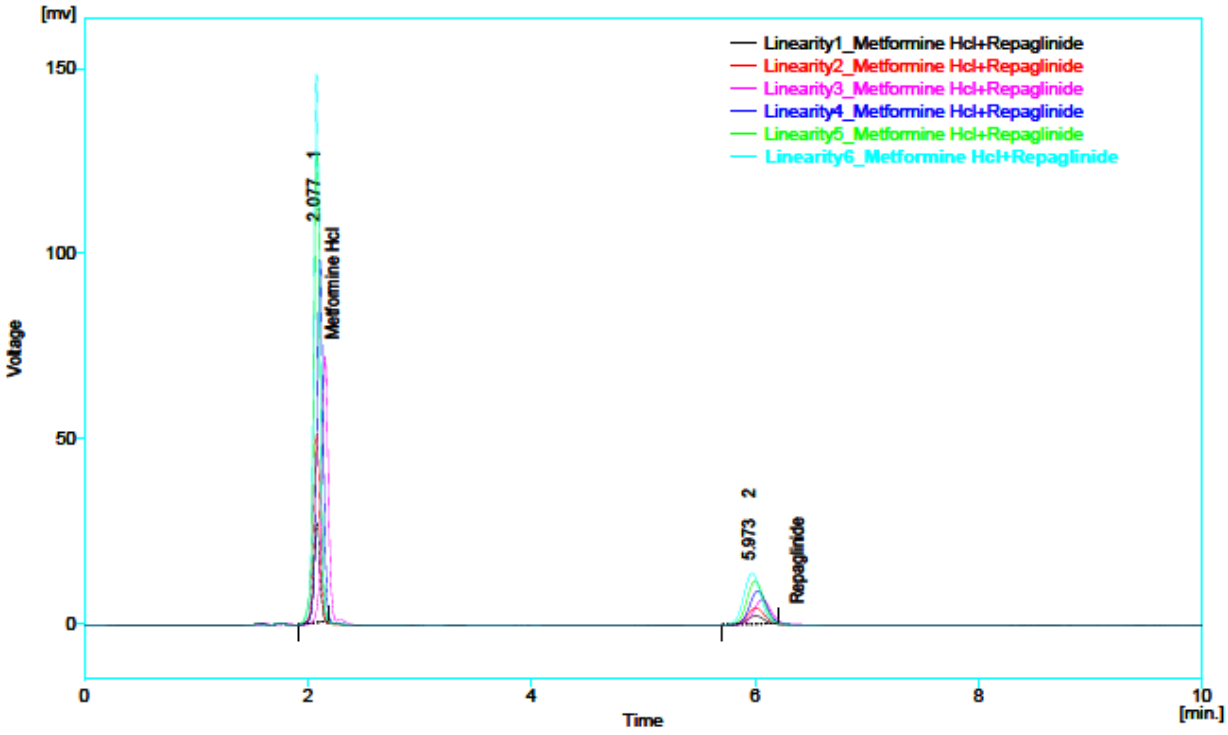
*Column Performance Table (From 50% - Linearity6_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.077 | 0.063 | 1.118 | 5956 | - |
| 2 | 5.973 | 0.167 | 1.116 | 7116 | 19.938 |

**FIGURE – 18**

## LINERARITY CHROMATOGRAM– I-IV

## METFORMIN HYDROCHLORIDE (150 µg) AND REPAGLINIDE (0.6 µg)




*Result Table (Uncal - Linearity6_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.077 | 599.490 | 148.061 | 79.959 |
| 2 | 5.973 | 147.491 | 13.774 | 20.041 |
| | Total | 746.981 | 161.835 | 100.000 |

*Column Performance Table (From 50% - Linearity6_Metformine Hcl+Repaglinide)*

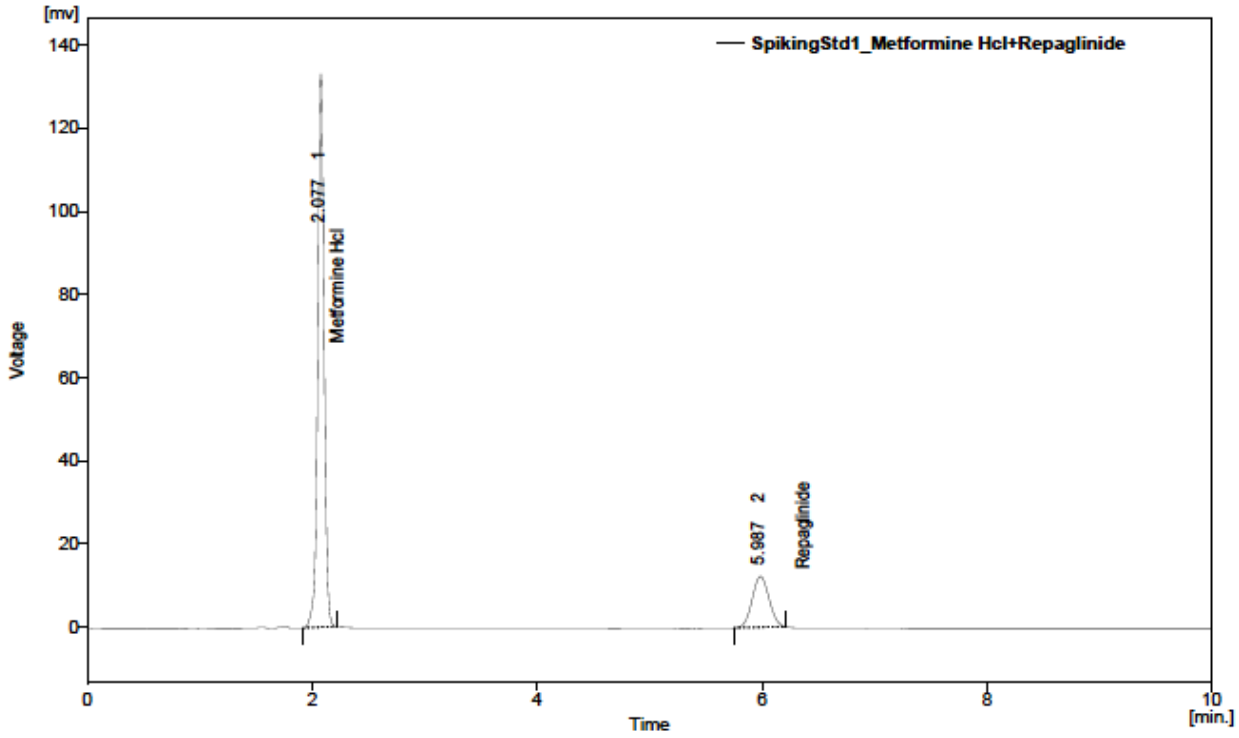
| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.077 | 0.063 | 1.118 | 5956 | - |
| 2 | 5.973 | 0.167 | 1.116 | 7116 | 19.938 |

## ACCURACY

**FIGURE – 19**

## SPIKING STANDARD CHROMATOGRAM – I

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - SpikingStd1_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.077 | 521.713 | 132.846 | 80.329 |
| 2 | 5.987 | 127.754 | 12.259 | 19.671 |
| | Total | 649.467 | 145.105 | 100.000 |

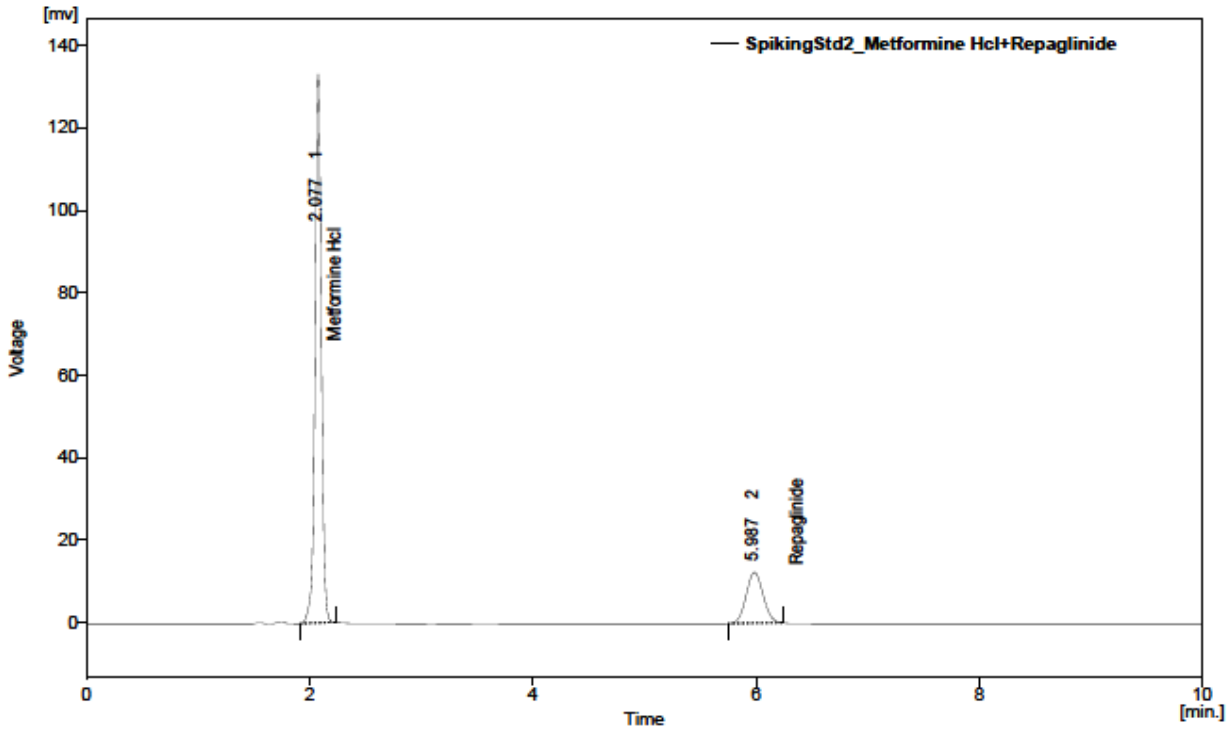
*Column Performance Table (From 50% - SpikingStd1_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.077 | 0.063 | 1.059 | 5956 | - |
| 2 | 5.987 | 0.170 | 1.068 | 6870 | 19.721 |

**FIGURE – 20**

## SPIKING STANDARD CHROMATOGRAM – II

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - SpikingStd2_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.077 | 522.603 | 132.896 | 80.093 |
| 2 | 5.987 | 129.896 | 12.334 | 19.907 |
| | Total | 652.499 | 145.230 | 100.000 |

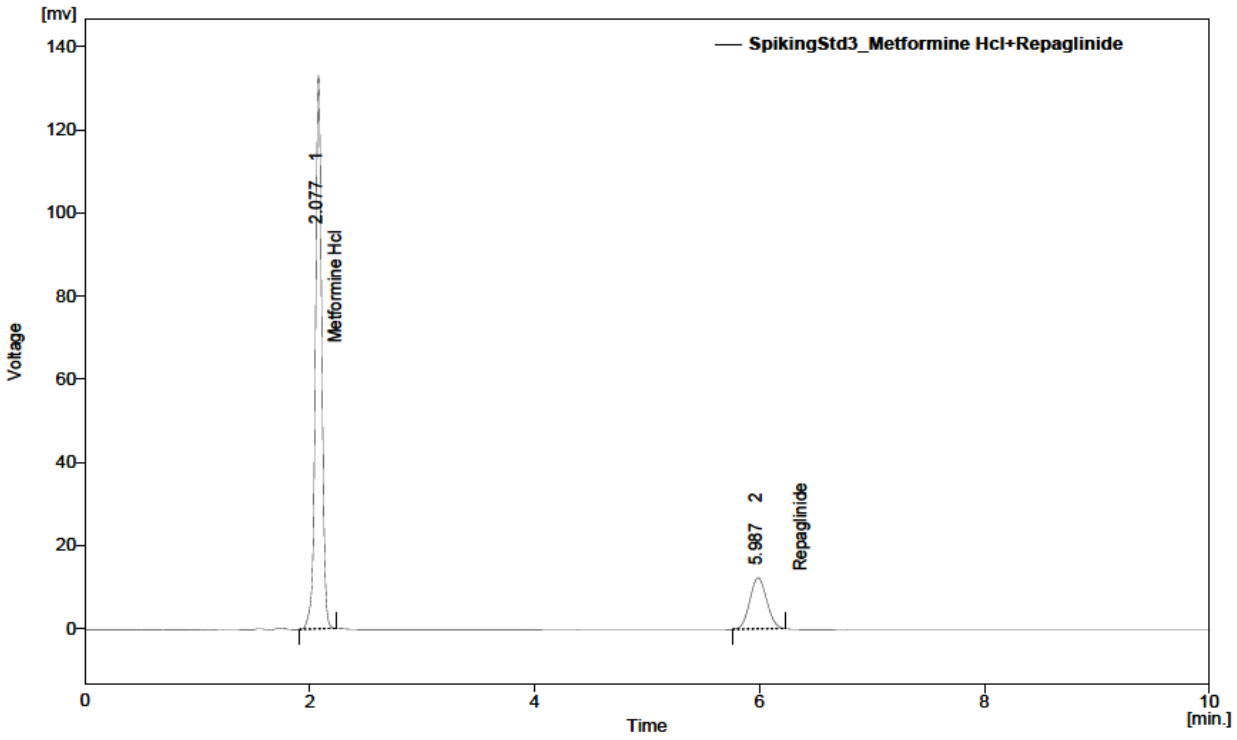
*Column Performance Table (From 50% - SpikingStd2_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.077 | 0.063 | 1.059 | 5956 | - |
| 2 | 5.987 | 0.170 | 1.091 | 6870 | 19.721 |

**FIGURE – 21**

## SPIKING STANDARD CHROMATOGRAM – III

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - SpikingStd3_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.077 | 522.248 | 132.986 | 80.262 |
| 2 | 5.987 | 128.434 | 12.309 | 19.738 |
| | Total | 650.682 | 145.295 | 100.000 |

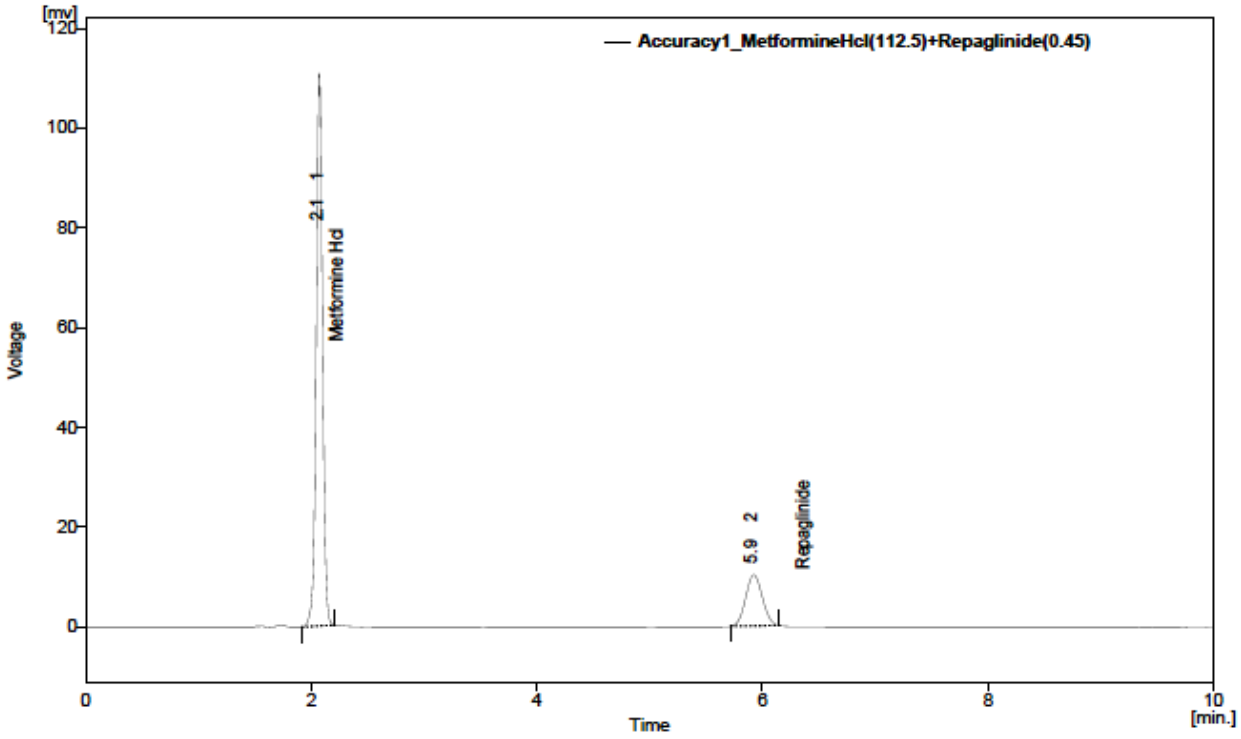
*Column Performance Table (From 50% - SpikingStd3_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.077 | 0.063 | 1.059 | 5956 | - |
| 2 | 5.987 | 0.167 | 1.093 | 7148 | 20.007 |

**FIGURE – 22**

**ACCURACY CHROMATOGRAM –TRIAL I (Solution-1)**

**METFORMIN HYDROCHLORIDE (112.5 µg) AND REPAGLINIDE (0.45 µg)**




*Result Table (Uncal - Accuracy1_MetformineHcl(112.5)+Repaglinide(0.45))*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.070 | 433.646 | 110.826 | 80.408 |
| 2 | 5.923 | 105.662 | 10.277 | 19.592 |
| | Total | 539.309 | 121.103 | 100.000 |

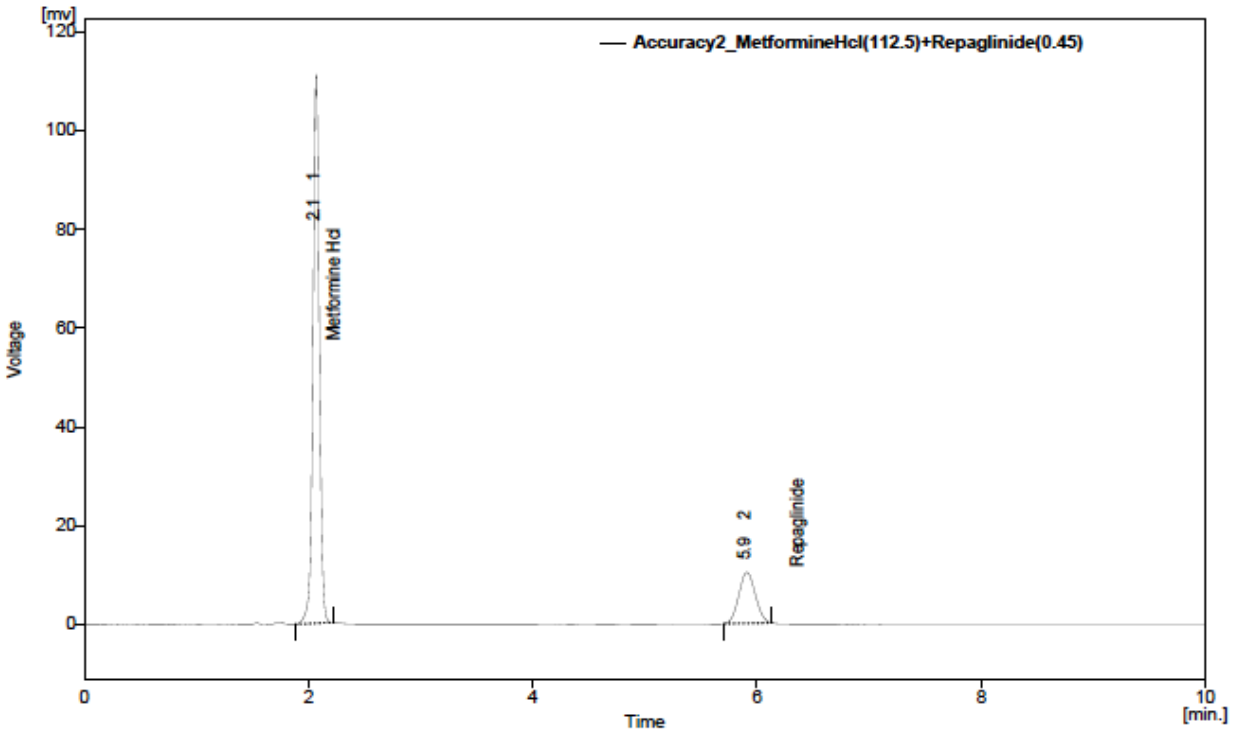
*Column Performance Table (From 50% - Accuracy1_MetformineHcl(112.5)+Repaglinide(0.45))*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.070 | 0.063 | 1.118 | 5918 | - |
| 2 | 5.923 | 0.163 | 1.143 | 7286 | 20.007 |

**FIGURE – 23**

## ACCURACY CHROMATOGRAM –TRIAL II (Solution-1)

## METFORMIN HYDROCHLORIDE (112.5 µg) AND REPAGLINIDE (0.45 µg)




*Result Table (Uncal - Accuracy2_MetformineHcl(112.5)+Repaglinide(0.45))*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.067 | 437.646 | 111.175 | 80.461 |
| 2 | 5.910 | 106.275 | 10.331 | 19.539 |
| | Total | 543.922 | 121.507 | 100.000 |

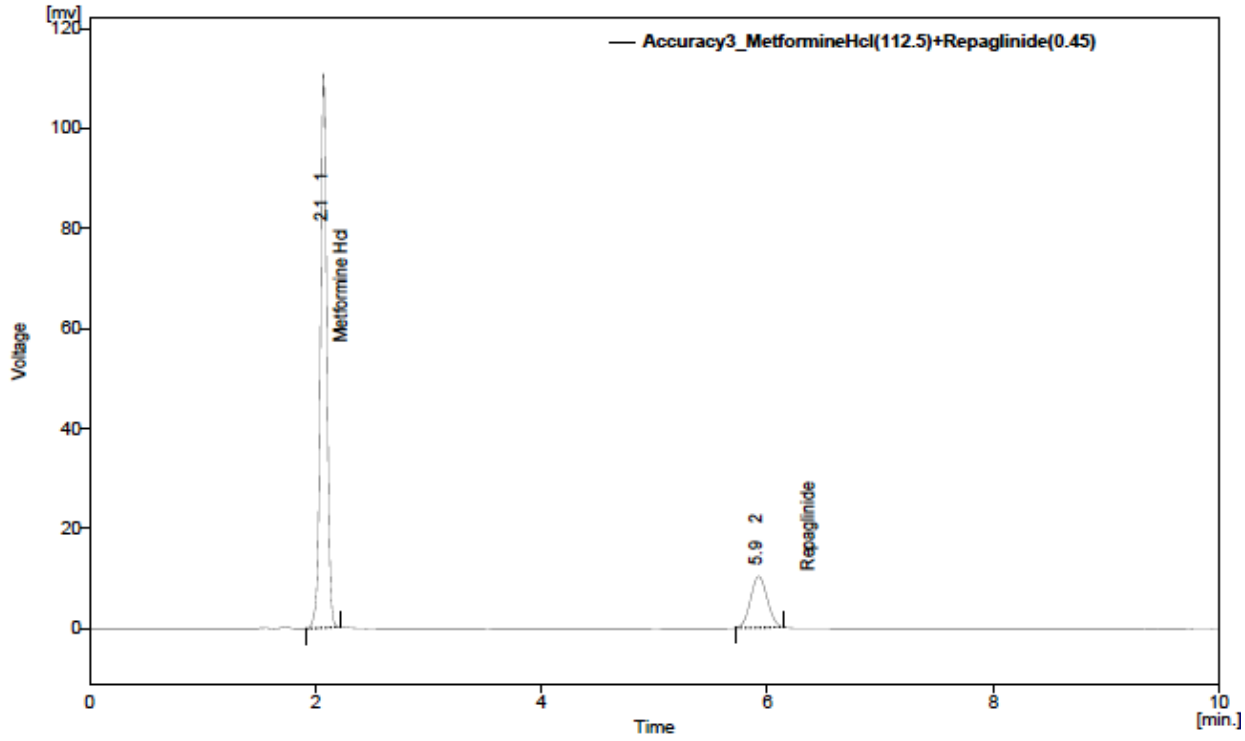
*Column Performance Table (From 50% - Accuracy2_MetformineHcl(112.5)+Repaglinide(0.45))*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.067 | 0.063 | 1.118 | 5899 | - |
| 2 | 5.910 | 0.167 | 1.143 | 6966 | 19.666 |

**FIGURE – 24**

## ACCURACY CHROMATOGRAM – TRIAL III (Solution-1)

## METFORMIN HYDROCHLORIDE (112.5 µg) AND REPAGLINIDE (0.45 µg)




*Result Table (Uncal - Accuracy3_MetformineHcl(112.5)+Repaglinide(0.45))*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.070 | 434.123 | 110.855 | 80.425 |
| 2 | 5.923 | 105.662 | 10.277 | 19.575 |
| | Total | 539.786 | 121.132 | 100.000 |

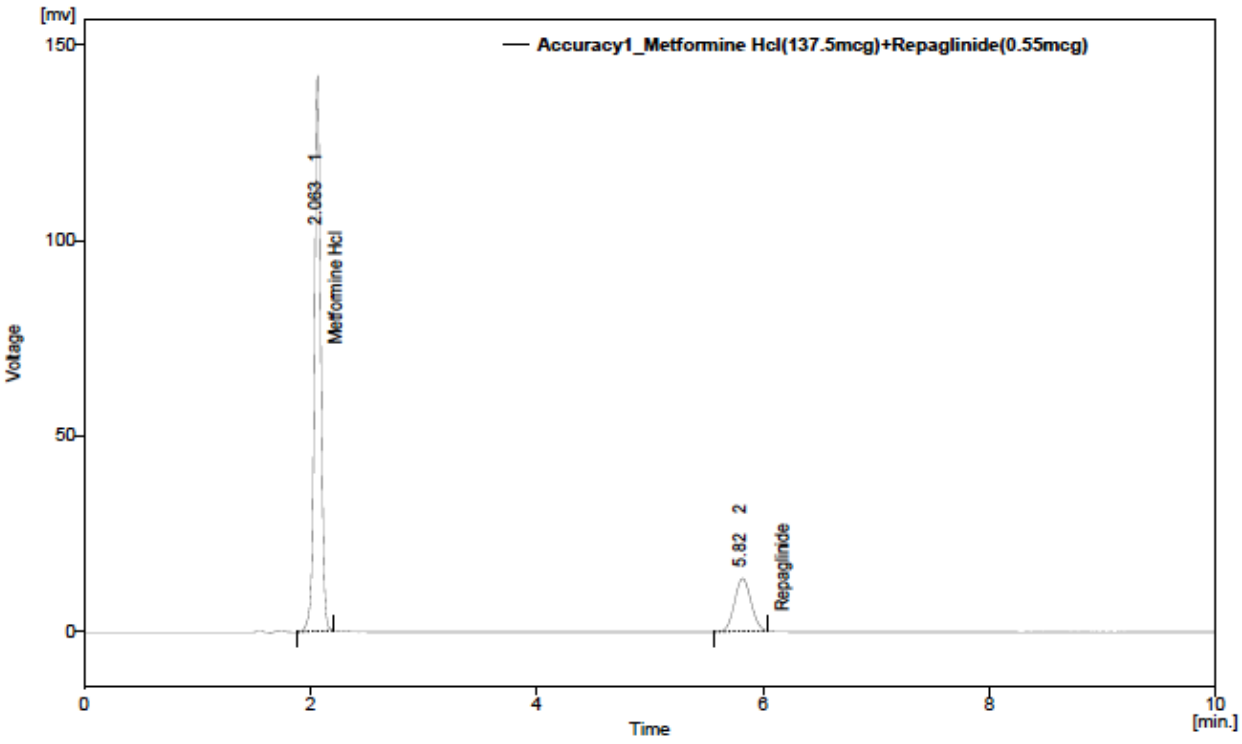
*Column Performance Table (From 50% - Accuracy3_MetformineHcl(112.5)+Repaglinide(0.45))*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.070 | 0.063 | 1.118 | 5918 | - |
| 2 | 5.923 | 0.163 | 1.143 | 7286 | 20.007 |

**FIGURE – 25**

## ACCURACY CHROMATOGRAM – TRIAL I (Solution-2)

## METFORMIN HYDROCHLORIDE (137.5 µg) AND REPAGLINIDE (0.55 µg)




*Result Table (Uncal - Accuracy1_Metformine Hcl(137.5mcg)+Repaglinide(0.55mcg))*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.063 | 572.031 | 141.987 | 80.201 |
| 2 | 5.820 | 140.278 | 13.505 | 19.799 |
| | Total | 712.309 | 155.493 | 100.000 |

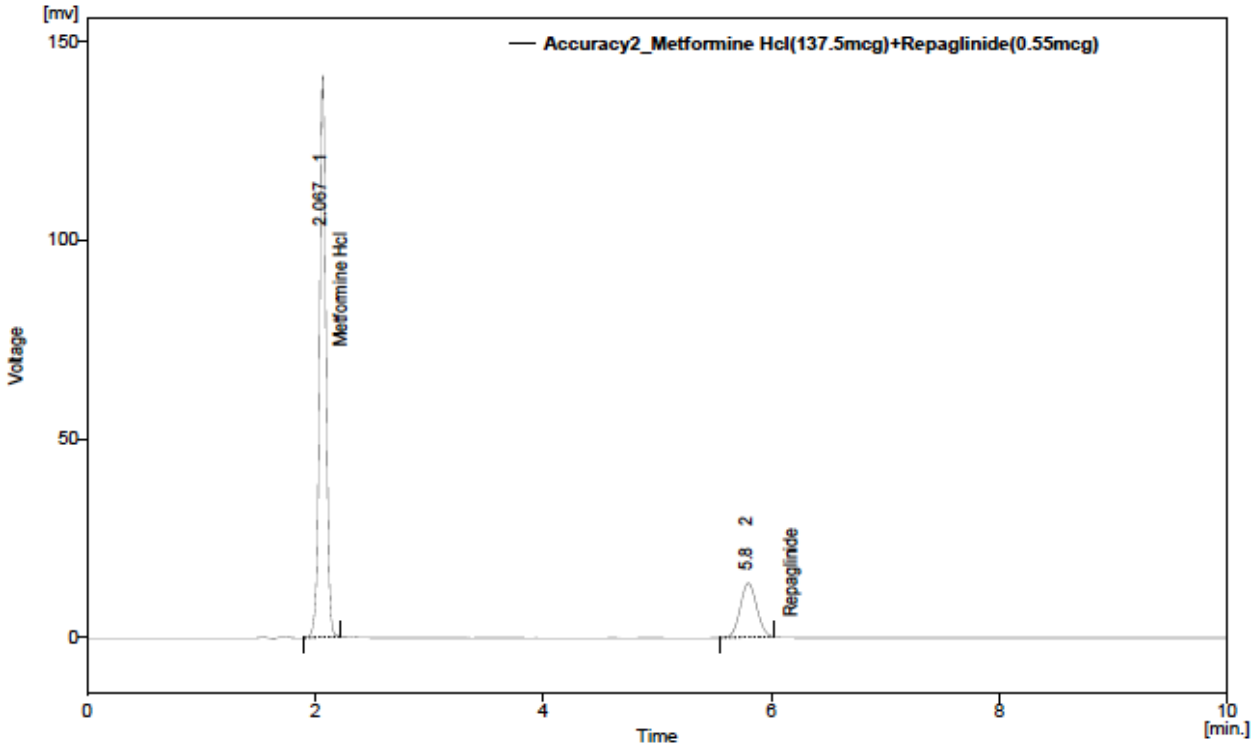
*Column Performance Table (From 50% - Accuracy1_Metformine Hcl(137.5mcg)+Repaglinide(0.55mcg))*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.063 | 0.063 | 1.059 | 5880 | - |
| 2 | 5.820 | 0.160 | 1.095 | 7330 | 19.796 |

**FIGURE – 26**

## ACCURACY CHROMATOGRAM – TRIAL II (Solution-2)

## METFORMIN HYDROCHLORIDE (137.5 µg) AND REPAGLINIDE (0.55 µg)




*Result Table (Uncal - Accuracy2_Metformine Hcl(137.5mcg)+Repaglinide(0.55mcg))*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.067 | 574.675 | 141.571 | 80.181 |
| 2 | 5.800 | 140.101 | 13.616 | 19.819 |
| | Total | 714.776 | 155.186 | 100.000 |

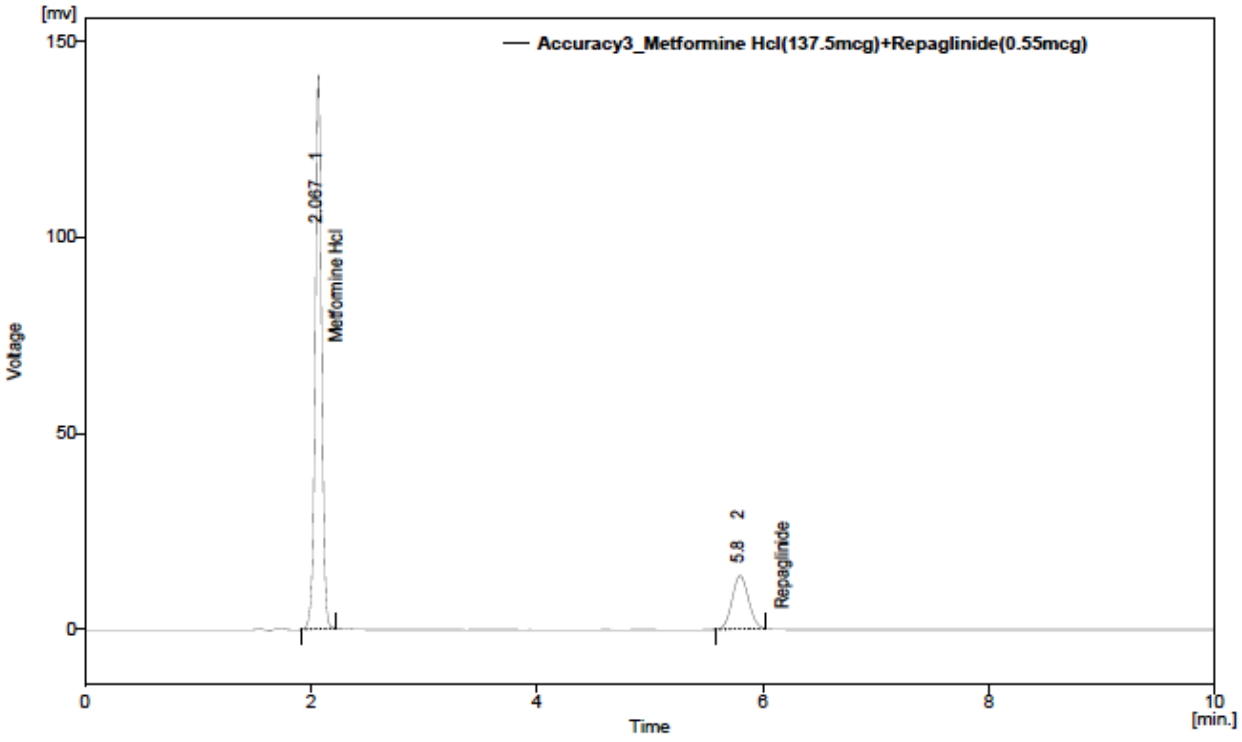
*Column Performance Table (From 50% - Accuracy2_Metformine Hcl(137.5mcg)+Repaglinide(0.55mcg))*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.067 | 0.060 | 1.059 | 6573 | - |
| 2 | 5.800 | 0.163 | 1.095 | 6986 | 19.673 |

**FIGURE – 27**

**ACCURACY CHROMATOGRAM – TRIAL III (Solution-2)**

**METFORMIN HYDROCHLORIDE (137.5 µg) AND REPAGLINIDE (0.55 µg)**




*Result Table (Uncal - Accuracy3_Metformine Hcl(137.5mcg)+Repaglinide(0.55mcg))*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.067 | 574.602 | 141.567 | 80.211 |
| 2 | 5.800 | 143.825 | 13.606 | 19.789 |
| | Total | 718.427 | 155.172 | 100.000 |

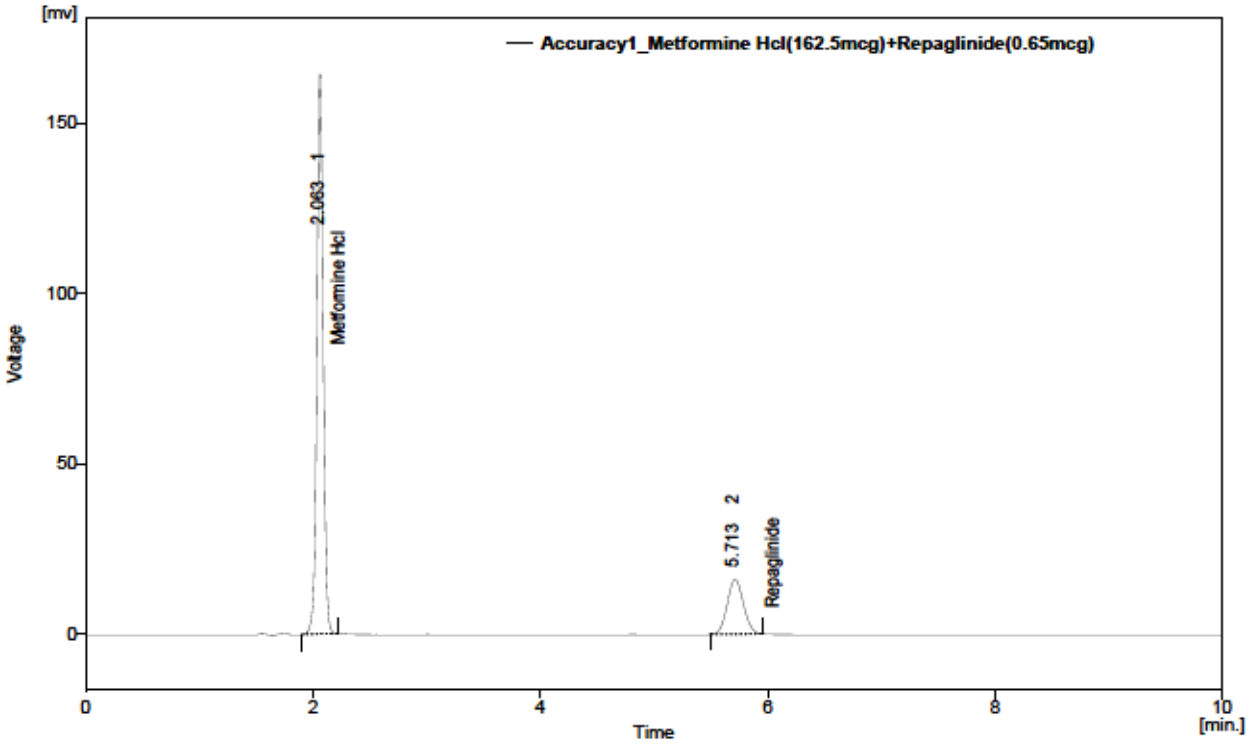
*Column Performance Table (From 50% - Accuracy3_Metformine Hcl(137.5mcg)+Repaglinide(0.55mcg))*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.067 | 0.060 | 1.059 | 6573 | - |
| 2 | 5.800 | 0.163 | 1.095 | 6986 | 19.673 |

**FIGURE – 28**

## ACCURACY CHROMATOGRAM – TRIAL I (Solution-3)

## METFORMIN HYDROCHLORIDE (162.5 µg) AND REPAGLINIDE (0.65 µg)




*Result Table (Uncal - Accuracy1_Metformine Hcl(162.5mcg)+Repaglinide(0.65mcg))*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.063 | 678.915 | 164.222 | 79.830 |
| 2 | 5.713 | 169.913 | 16.101 | 20.170 |
| | Total | 848.828 | 180.323 | 100.000 |

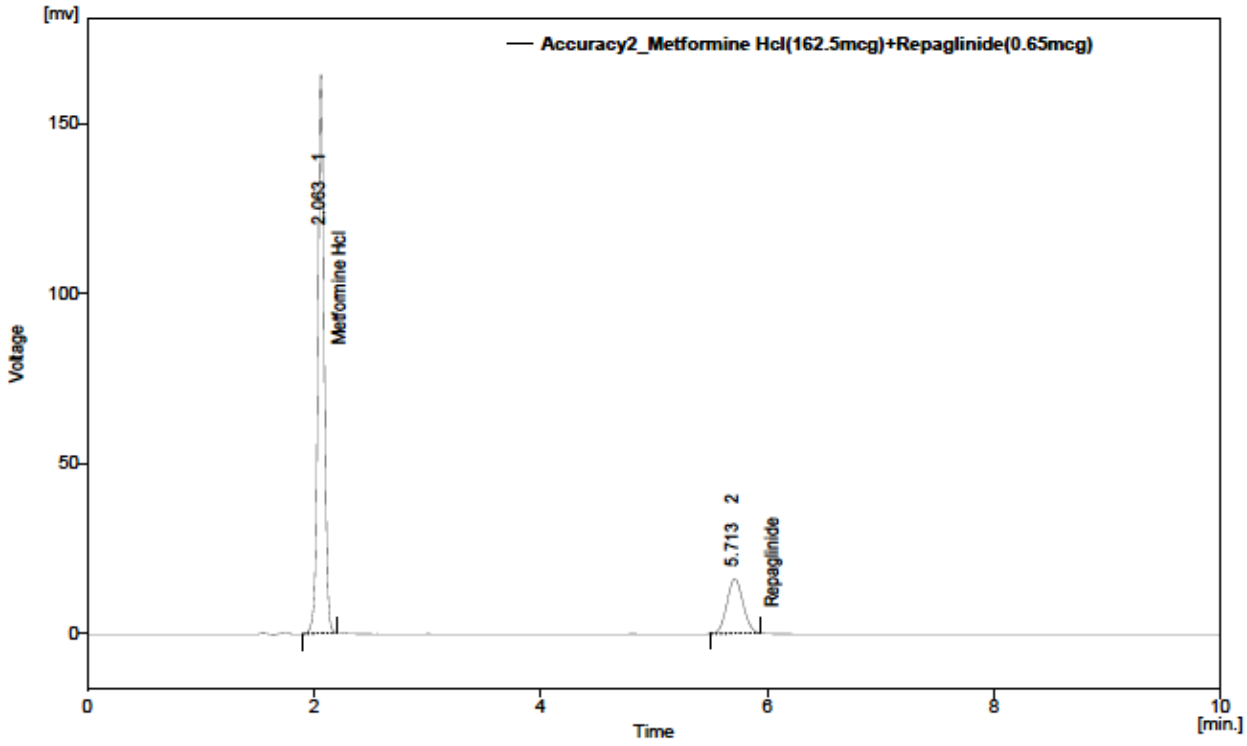
*Column Performance Table (From 50% - Accuracy1_Metformine Hcl(162.5mcg)+Repaglinide(0.65mcg))*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.063 | 0.060 | 1.059 | 6552 | - |
| 2 | 5.713 | 0.160 | 1.122 | 7064 | 19.525 |

**FIGURE – 29**

## ACCURACY CHROMATOGRAM – TRIAL II (Solution-3)

## METFORMIN HYDROCHLORIDE (162.5 µg) AND REPAGLINIDE (0.65 µg)




*Result Table (Uncal - Accuracy2_Metformine Hcl(162.5mcg)+Repaglinide(0.65mcg))*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.063 | 678.376 | 164.192 | 79.997 |
| 2 | 5.713 | 168.128 | 16.038 | 20.003 |
| | Total | 846.504 | 180.230 | 100.000 |

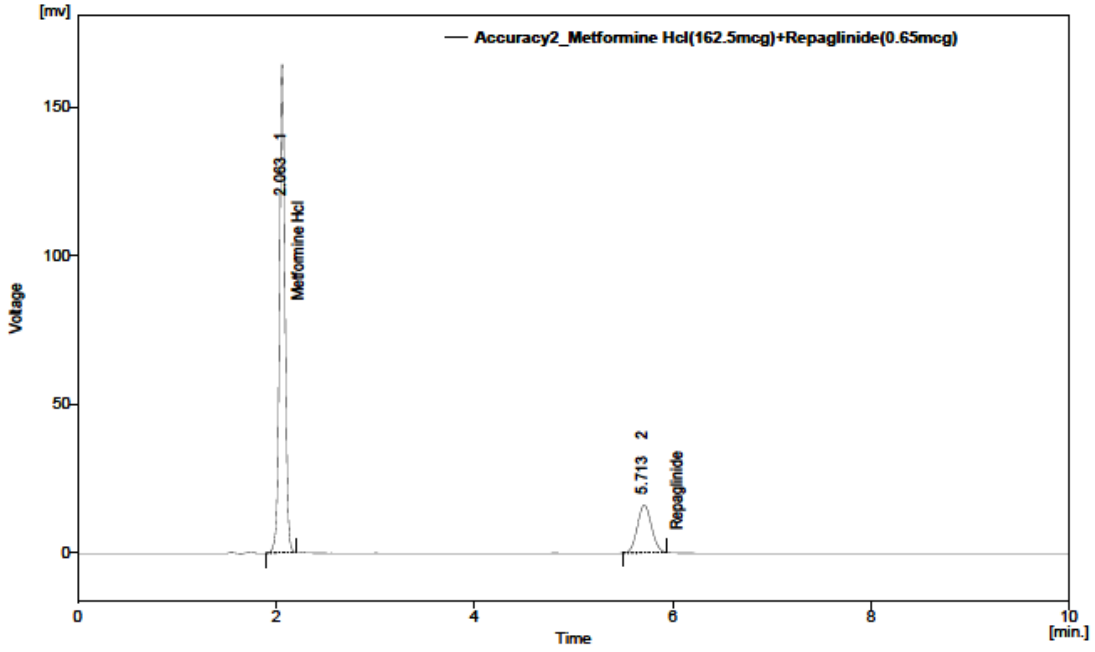
*Column Performance Table (From 50% - Accuracy2_Metformine Hcl(162.5mcg)+Repaglinide(0.65mcg))*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.063 | 0.060 | 1.059 | 6552 | - |
| 2 | 5.713 | 0.160 | 1.098 | 7064 | 19.525 |

**FIGURE – 30**

**ACCURACY CHROMATOGRAM – TRIAL III (Solution-3)**

**METFORMIN HYDROCHLORIDE (162.5 µg) AND REPAGLINIDE (0.65 µg)**




*Result Table (Uncal - Accuracy2_Metformine Hcl(162.5mcg)+Repaglinide(0.65mcg))*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.063 | 678.376 | 164.192 | 79.997 |
| 2 | 5.713 | 168.128 | 16.038 | 20.003 |
| | Total | 846.504 | 180.230 | 100.000 |

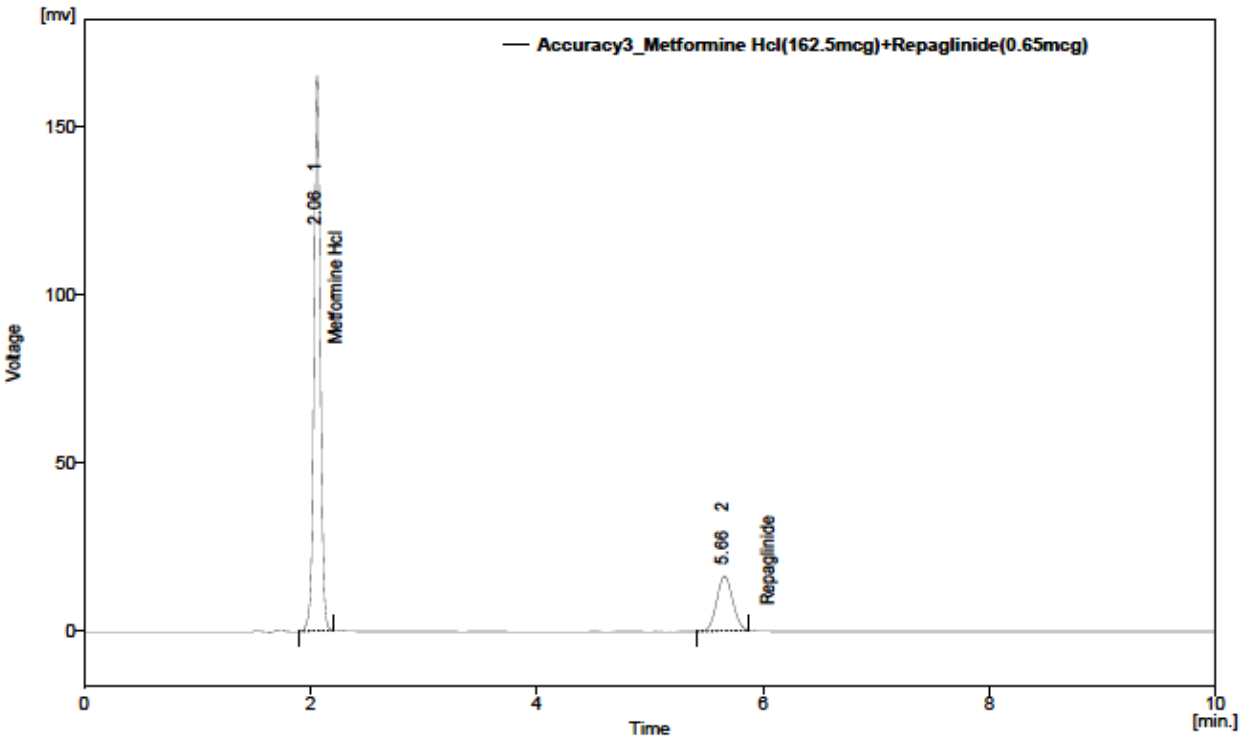
*Column Performance Table (From 50% - Accuracy2_Metformine Hcl(162.5mcg)+Repaglinide(0.65mcg))*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.063 | 0.060 | 1.059 | 6552 | - |
| 2 | 5.713 | 0.160 | 1.098 | 7064 | 19.525 |

**FIGURE – 31**

**ACCURACY CHROMATOGRAM – TRIAL IV (Solution -3)**

**METFORMIN HYDROCHLORIDE (162.5 µg) AND REPAGLINIDE (0.65 µg)**




*Result Table (Uncal - Accuracy3_Metformine Hcl(162.5mcg)+Repaglinide(0.65mcg))*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.060 | 677.029 | 165.225 | 80.046 |
| 2 | 5.660 | 165.794 | 16.306 | 19.954 |
| | Total | 842.823 | 181.531 | 100.000 |

*Column Performance Table (From 50% - Accuracy3_Metformine Hcl(162.5mcg)+Repaglinide(0.65mcg))*

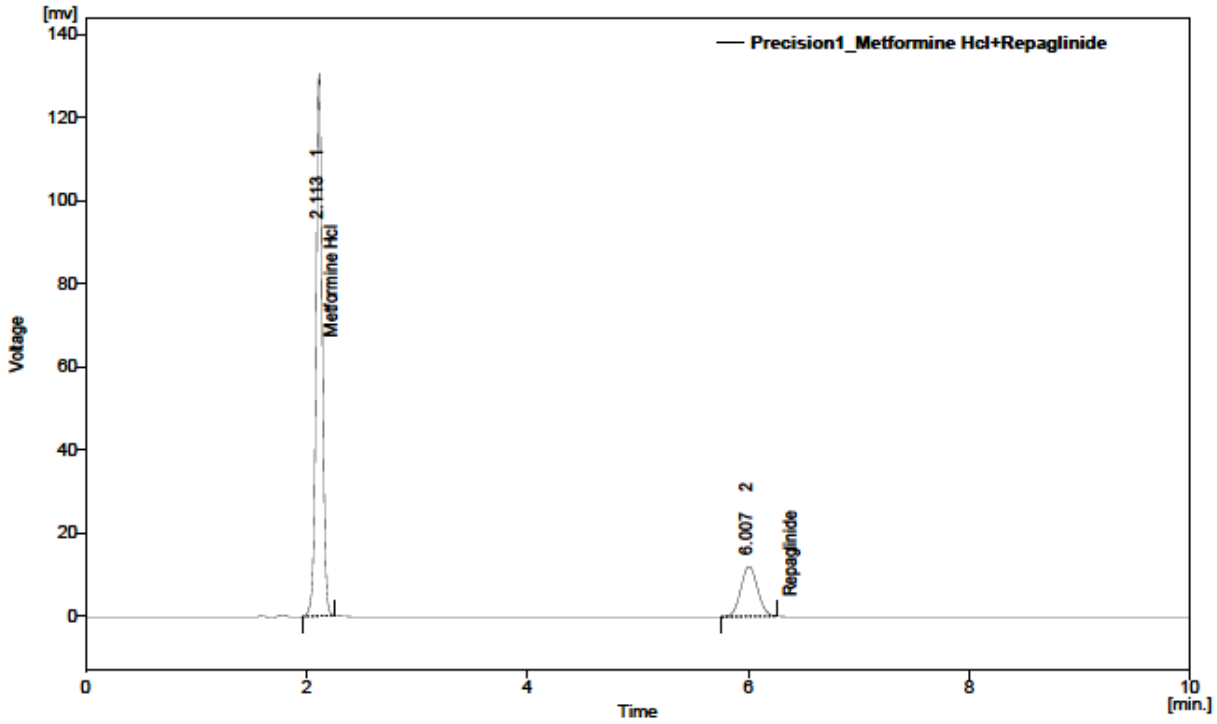
| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.060 | 0.060 | 1.059 | 6530 | - |
| 2 | 5.660 | 0.157 | 1.125 | 7231 | 19.554 |

## PRECISION

**FIGURE – 32**

## PRECISION CHROMATOGRAM– I

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - Precision1_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.113 | 510.122 | 130.627 | 79.988 |
| 2 | 6.007 | 126.374 | 12.089 | 20.012 |
| | Total | 636.496 | 142.717 | 100.000 |

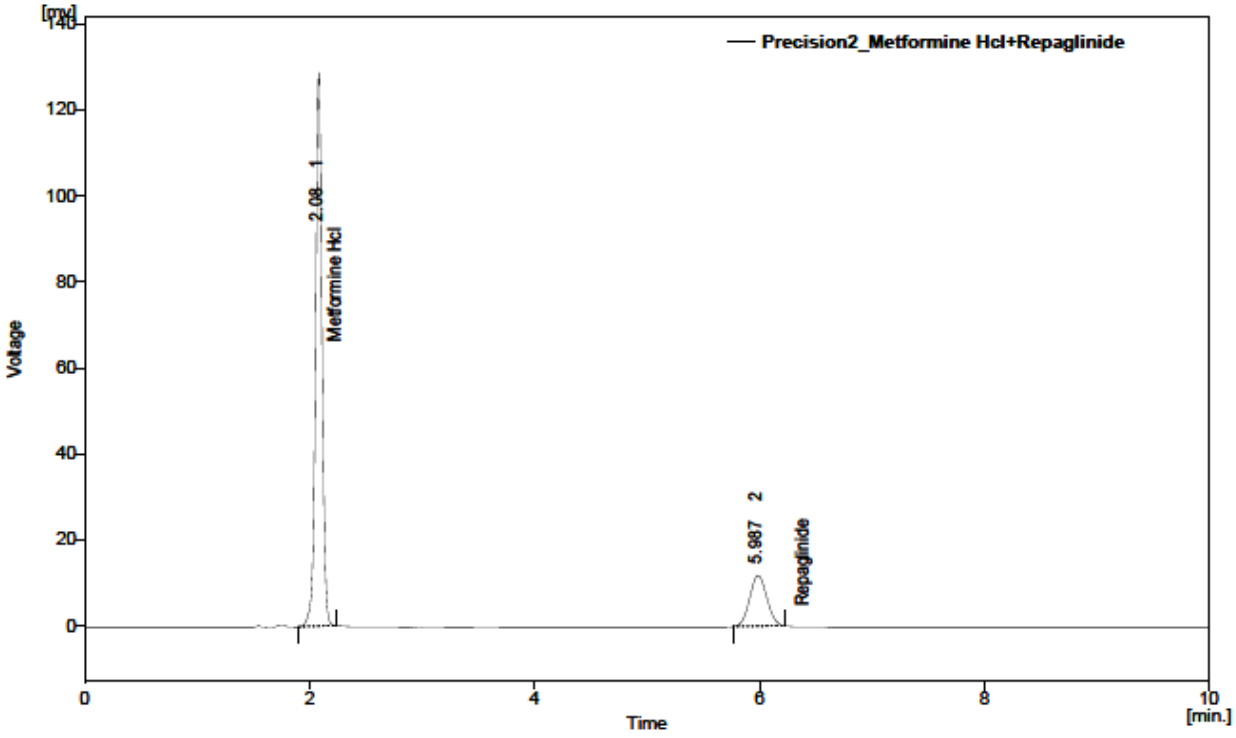
*Column Performance Table (From 50% - Precision1_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.113 | 0.063 | 1.059 | 6169 | - |
| 2 | 6.007 | 0.167 | 1.091 | 7196 | 19.921 |

**FIGURE – 33**

## PRECISION CHROMATOGRAM– II

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - Precision2_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.080 | 511.699 | 128.448 | 80.344 |
| 2 | 5.987 | 125.187 | 11.782 | 19.656 |
| | Total | 636.887 | 140.230 | 100.000 |

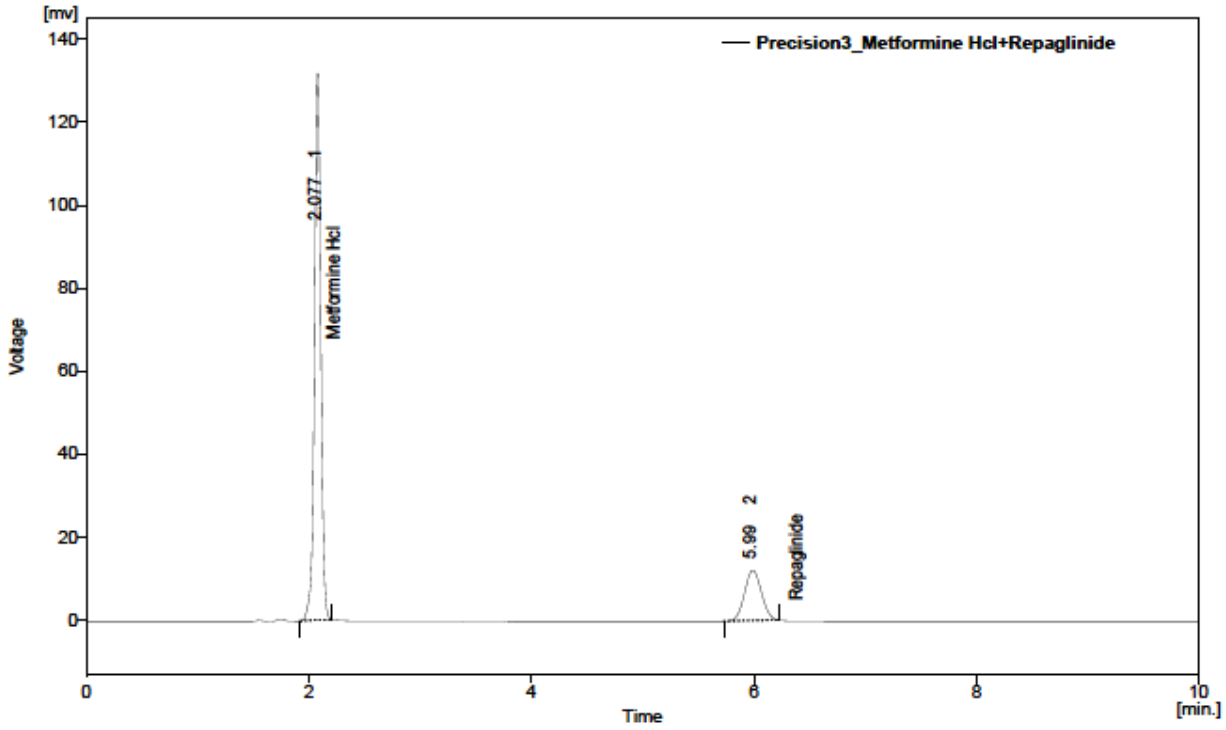
*Column Performance Table (From 50% - Precision2_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.080 | 0.063 | 1.118 | 5975 | - |
| 2 | 5.987 | 0.170 | 1.114 | 6870 | 19.704 |

**FIGURE – 34**

## PRECISION CHROMATOGRAM– III

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - Precision3_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.077 | 517.179 | 131.530 | 80.205 |
| 2 | 5.990 | 127.642 | 12.142 | 19.795 |
| | Total | 644.821 | 143.673 | 100.000 |

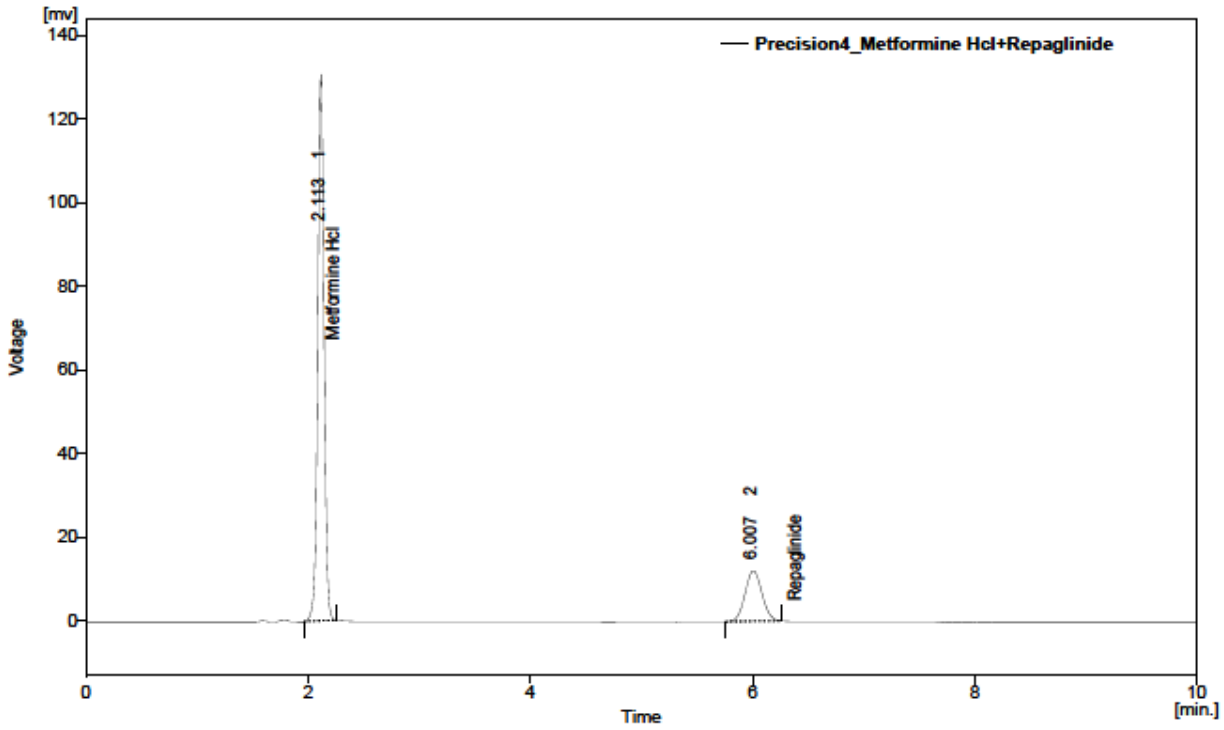
*Column Performance Table (From 50% - Precision3_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.077 | 0.060 | 1.118 | 6637 | - |
| 2 | 5.990 | 0.170 | 1.091 | 6878 | 20.024 |

**FIGURE – 35**

## PRECISION CHROMATOGRAM– IV

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - Precision4_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.113 | 509.128 | 130.627 | 79.988 |
| 2 | 6.007 | 127.376 | 12.089 | 20.012 |
| | Total | 636.504 | 142.717 | 100.000 |

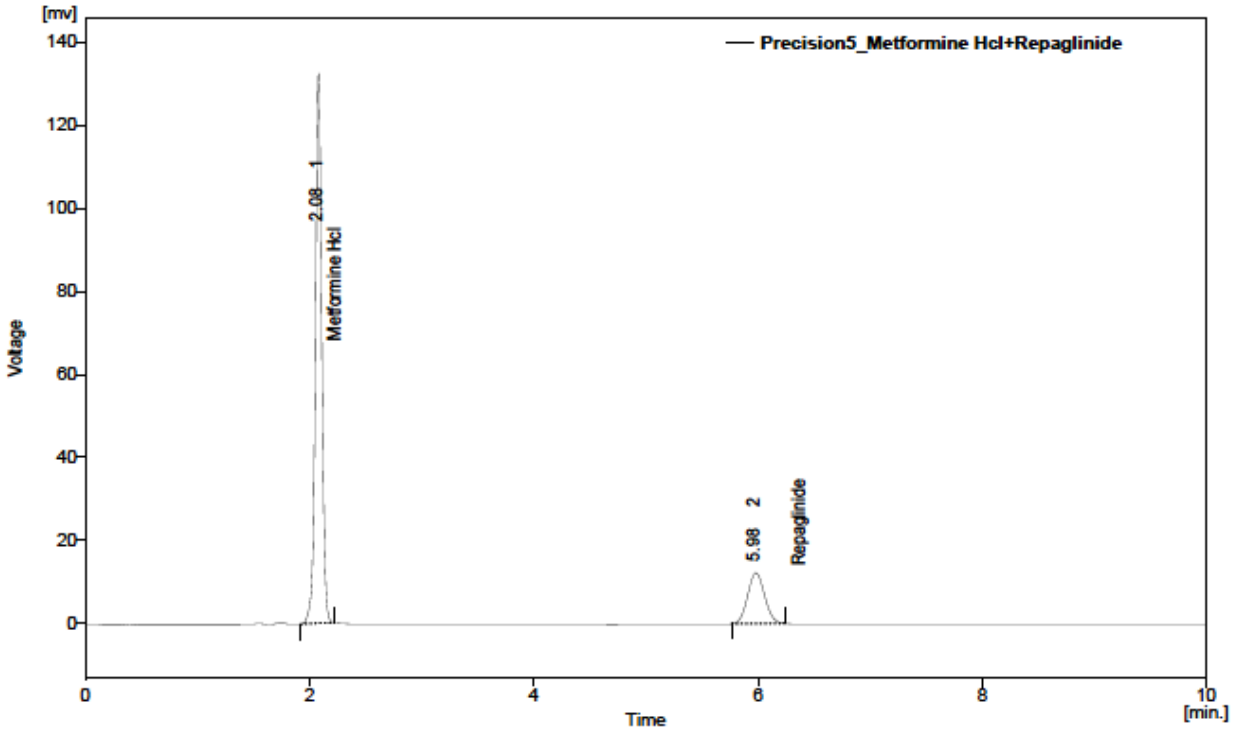
*Column Performance Table (From 50% - Precision4_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.113 | 0.063 | 1.059 | 6169 | - |
| 2 | 6.007 | 0.167 | 1.091 | 7196 | 19.921 |

**FIGURE – 36**

## PRECISION CHROMATOGRAM– V

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - Precision5_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.080 | 519.359 | 132.295 | 80.129 |
| 2 | 5.980 | 126.796 | 12.257 | 19.871 |
| | Total | 646.155 | 144.552 | 100.000 |

*Column Performance Table (From 50% - Precision5_Metformine Hcl+Repaglinide)*

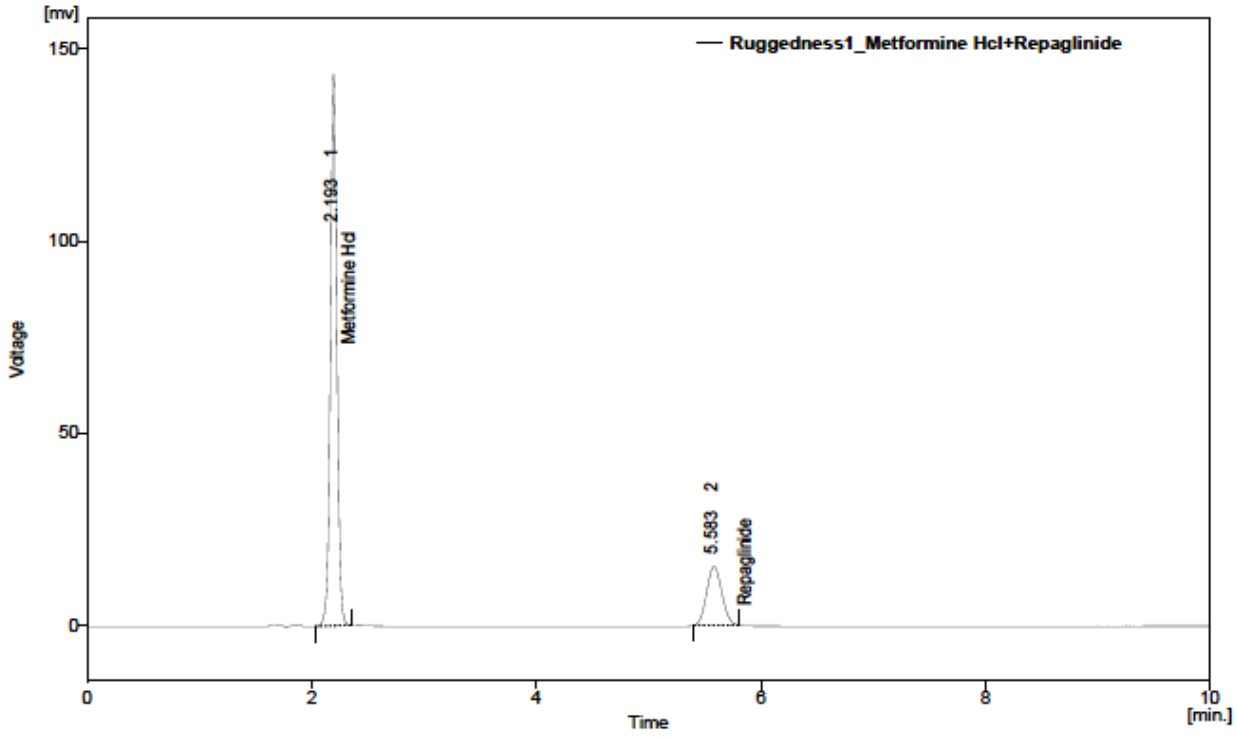
| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.080 | 0.060 | 1.059 | 6658 | - |
| 2 | 5.980 | 0.167 | 1.140 | 7132 | 20.249 |

## RUGGEDNESS

**FIGURE – 37**

## RUGGEDNESS CHROMATOGRAM– I

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - Ruggedness1_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.193 | 576.815 | 143.500 | 80.002 |
| 2 | 5.583 | 144.183 | 15.336 | 19.998 |
| | Total | 720.998 | 158.835 | 100.000 |

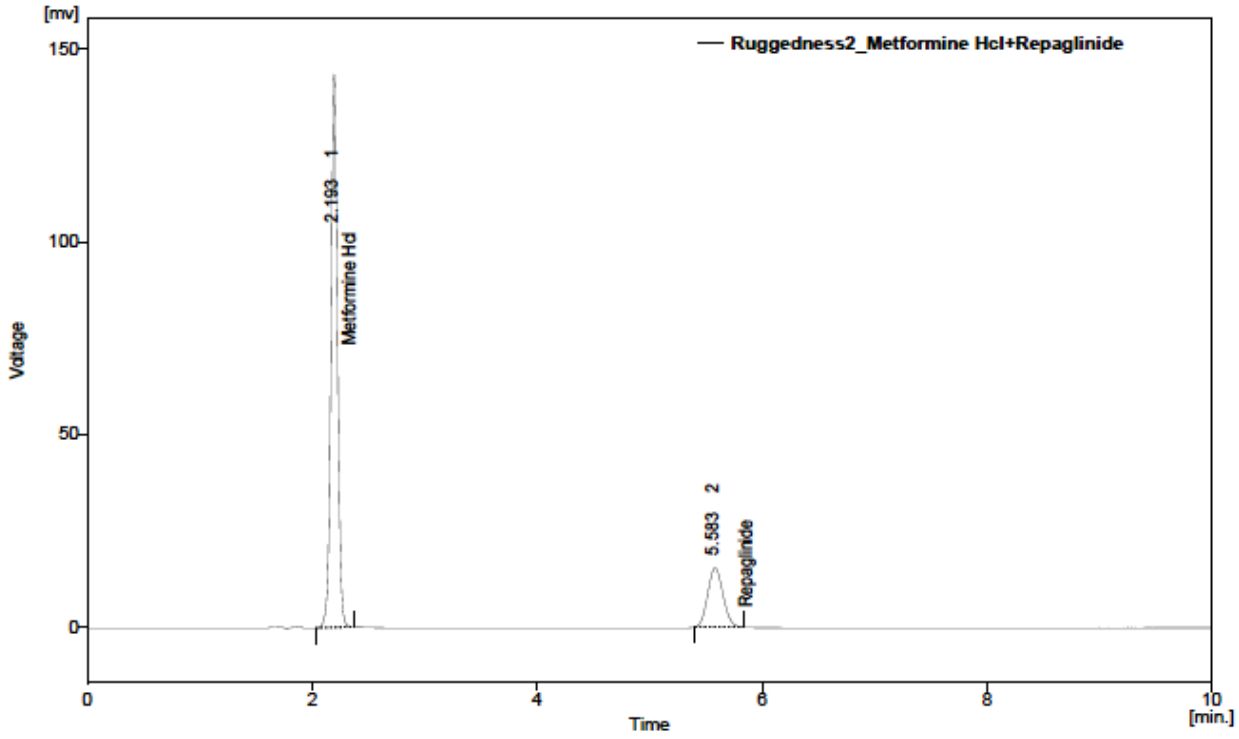
*Column Performance Table (From 50% - Ruggedness1_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.193 | 0.063 | 1.118 | 6644 | - |
| 2 | 5.583 | 0.150 | 1.128 | 7676 | 18.701 |

**FIGURE – 38**

## RUGGEDNESS CHROMATOGRAM– II

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - Ruggedness2_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.193 | 577.195 | 143.518 | 79.878 |
| 2 | 5.583 | 145.398 | 15.378 | 20.122 |
| | Total | 722.593 | 158.896 | 100.000 |

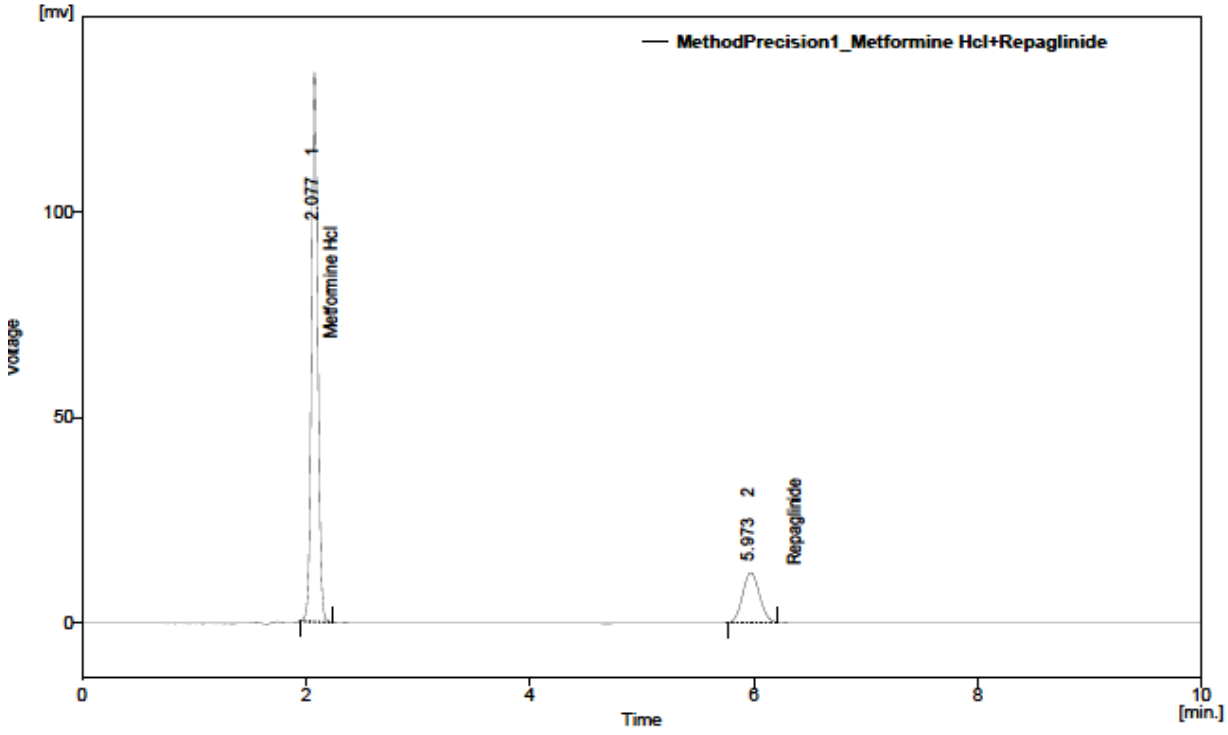
*Column Performance Table (From 50% - Ruggedness2_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.193 | 0.063 | 1.118 | 6644 | - |
| 2 | 5.583 | 0.150 | 1.128 | 7676 | 18.701 |

**FIGURE – 39**

## METHOD PRECISION CHROMATOGRAM– I

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - MethodPrecision1_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.077 | 516.525 | 133.863 | 80.209 |
| 2 | 5.973 | 127.447 | 12.219 | 19.791 |
| | Total | 643.972 | 146.082 | 100.000 |

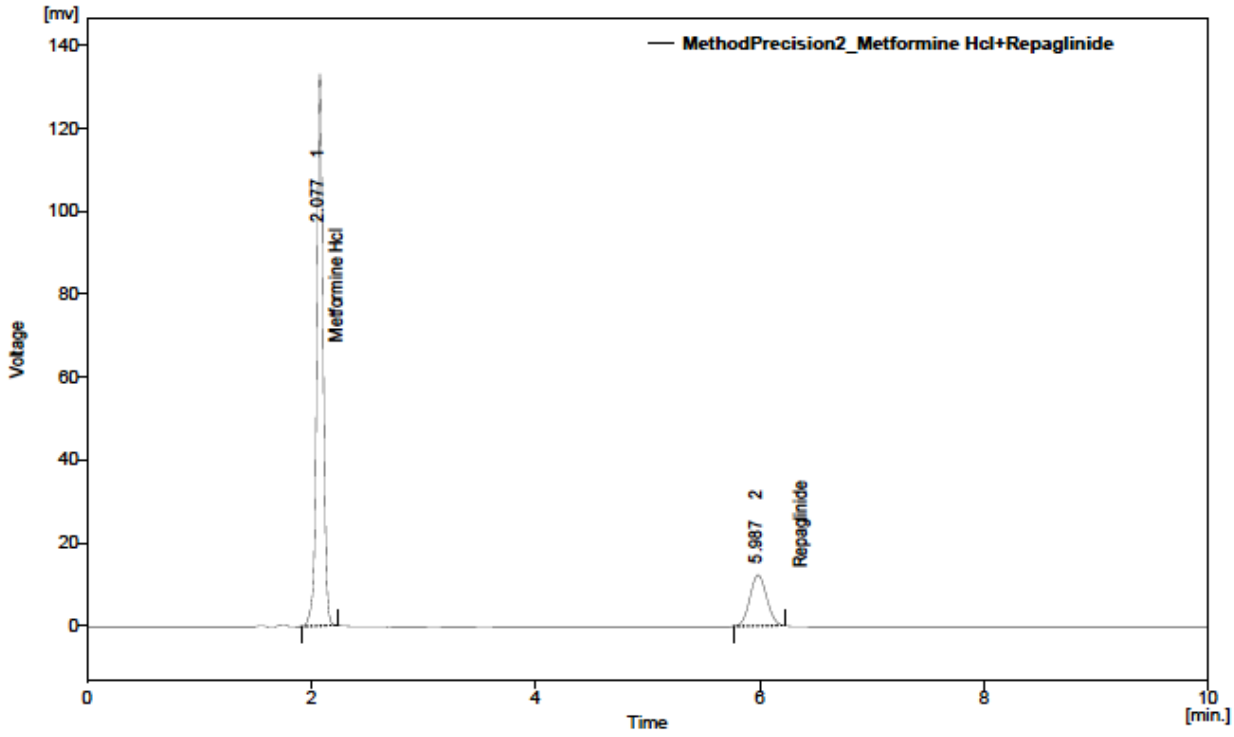
*Column Performance Table (From 50% - MethodPrecision1_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.077 | 0.063 | 1.125 | 5956 | - |
| 2 | 5.973 | 0.167 | 1.116 | 7116 | 19.938 |

**FIGURE – 40**

## METHOD PRECISION CHROMATOGRAM– II

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - MethodPrecision2_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.077 | 522.248 | 132.986 | 80.262 |
| 2 | 5.987 | 128.434 | 12.309 | 19.738 |
| | Total | 650.682 | 145.295 | 100.000 |

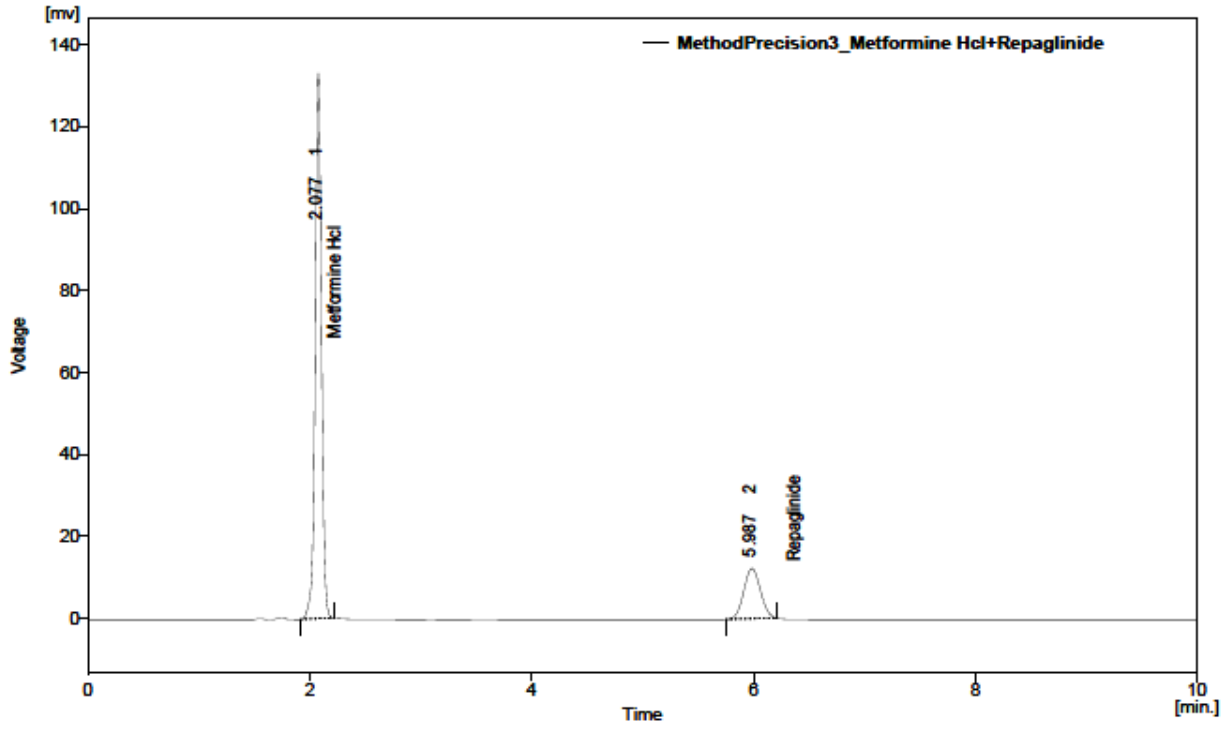
*Column Performance Table (From 50% - MethodPrecision2_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.077 | 0.063 | 1.059 | 5956 | - |
| 2 | 5.987 | 0.167 | 1.093 | 7148 | 20.007 |

**FIGURE – 41**

## METHOD PRECISION CHROMATOGRAM– III

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - MethodPrecision3_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.077 | 521.713 | 132.846 | 80.329 |
| 2 | 5.987 | 127.754 | 12.259 | 19.671 |
| | Total | 649.467 | 145.105 | 100.000 |

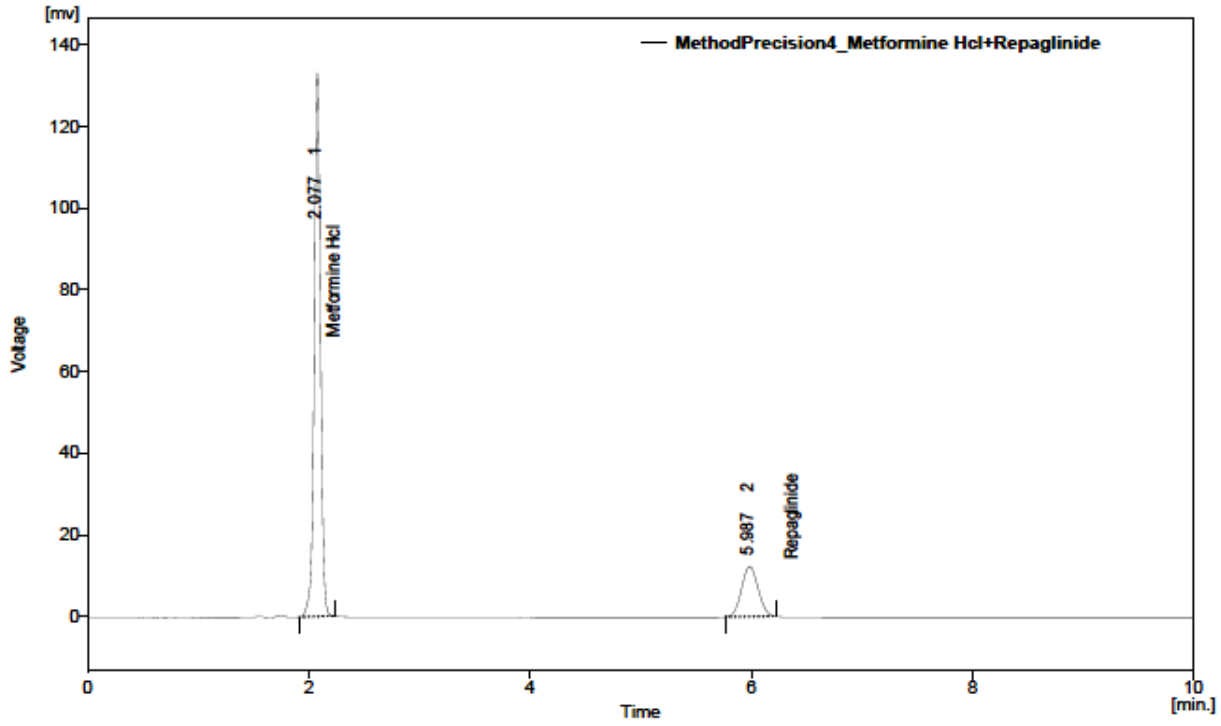
*Column Performance Table (From 50% - MethodPrecision3_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.077 | 0.063 | 1.059 | 5956 | - |
| 2 | 5.987 | 0.170 | 1.068 | 6870 | 19.721 |

**FIGURE – 42**

## METHOD PRECISION CHROMATOGRAM– IV

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - MethodPrecision4_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.077 | 521.242 | 132.986 | 80.262 |
| 2 | 5.987 | 127.430 | 12.309 | 19.738 |
| | Total | 648.672 | 145.295 | 100.000 |

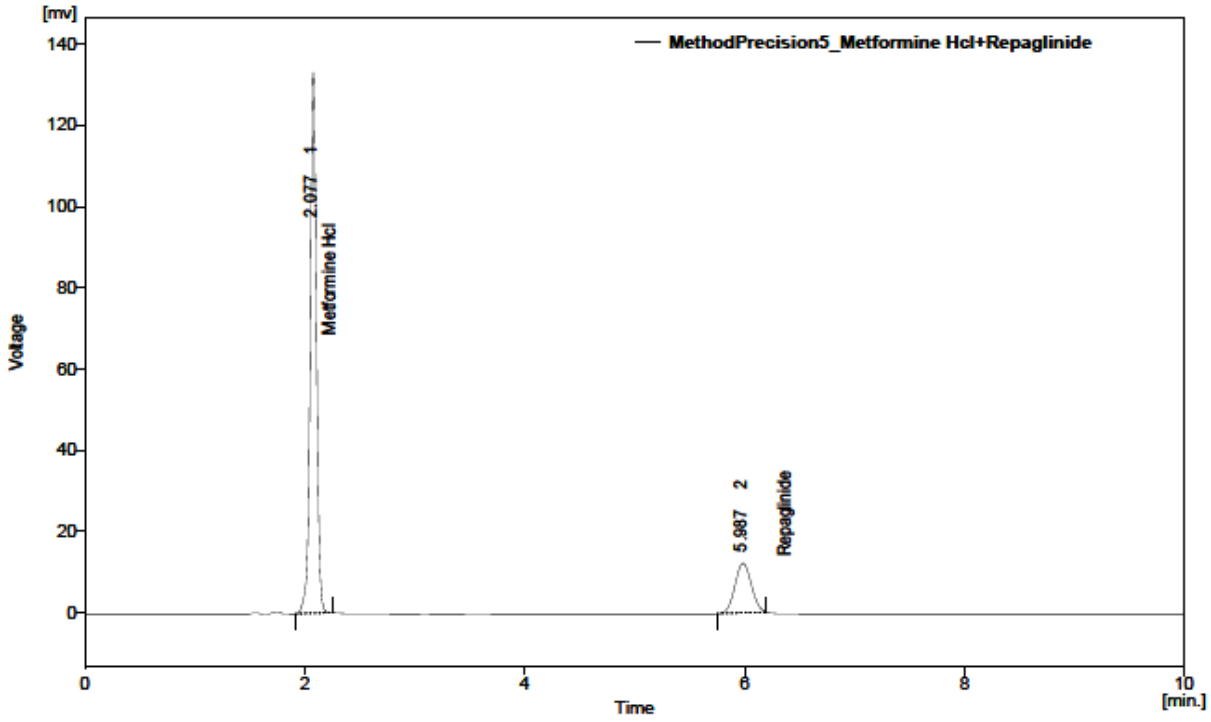
*Column Performance Table (From 50% - MethodPrecision4_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.077 | 0.063 | 1.059 | 5956 | - |
| 2 | 5.987 | 0.167 | 1.093 | 7148 | 20.007 |

**FIGURE – 43**

## METHOD PRECISION CHROMATOGRAM– V

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - MethodPrecision5_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.077 | 522.998 | 132.916 | 80.564 |
| 2 | 5.987 | 126.176 | 12.198 | 19.436 |
| | Total | 649.174 | 145.114 | 100.000 |

*Column Performance Table (From 50% - MethodPrecision5_Metformine Hcl+Repaglinide)*

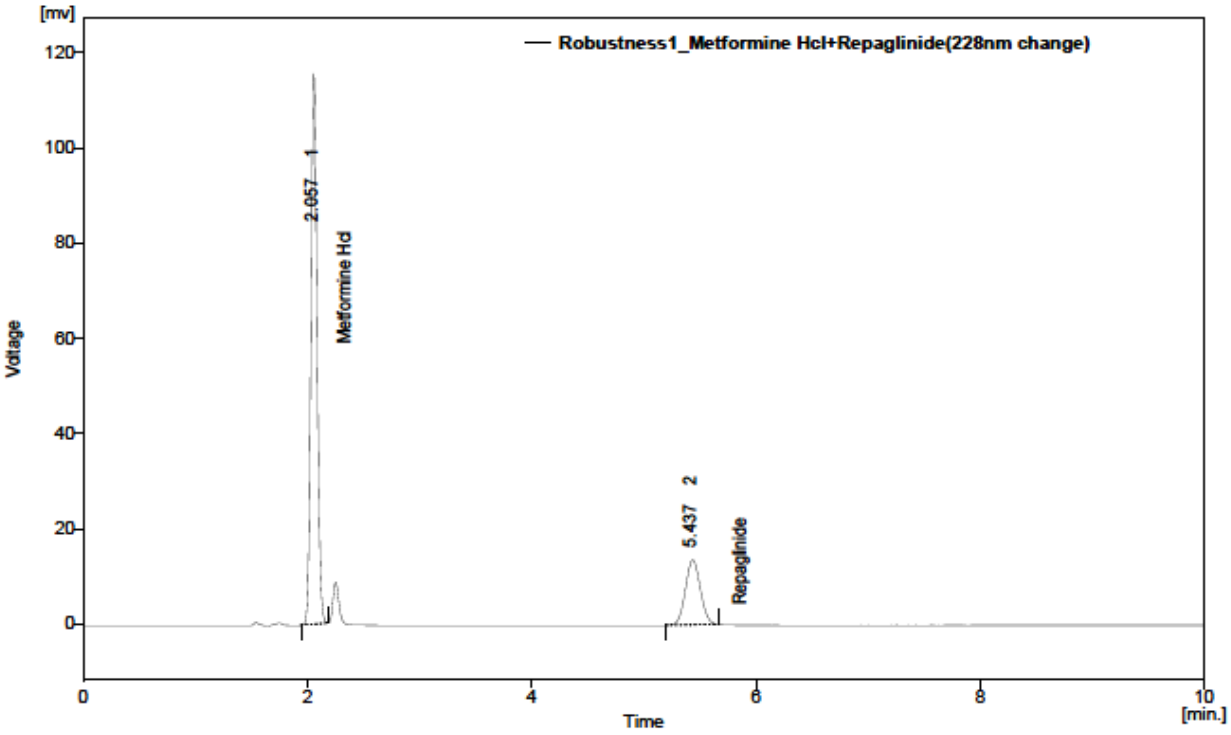
| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.077 | 0.063 | 1.059 | 5956 | - |
| 2 | 5.987 | 0.163 | 1.070 | 7443 | 20.301 |

## ROBUSTNESS

**FIGURE – 44**

## ROBUSTNESS CHROMATOGRAM– I

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg) AT 228 nm




*Result Table (Uncal - Robustness1_Metformine Hcl+Repaglinide(228nm change))*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.057 | 435.056 | 115.541 | 77.492 |
| 2 | 5.437 | 126.366 | 13.645 | 22.508 |
| | Total | 561.422 | 129.186 | 100.000 |

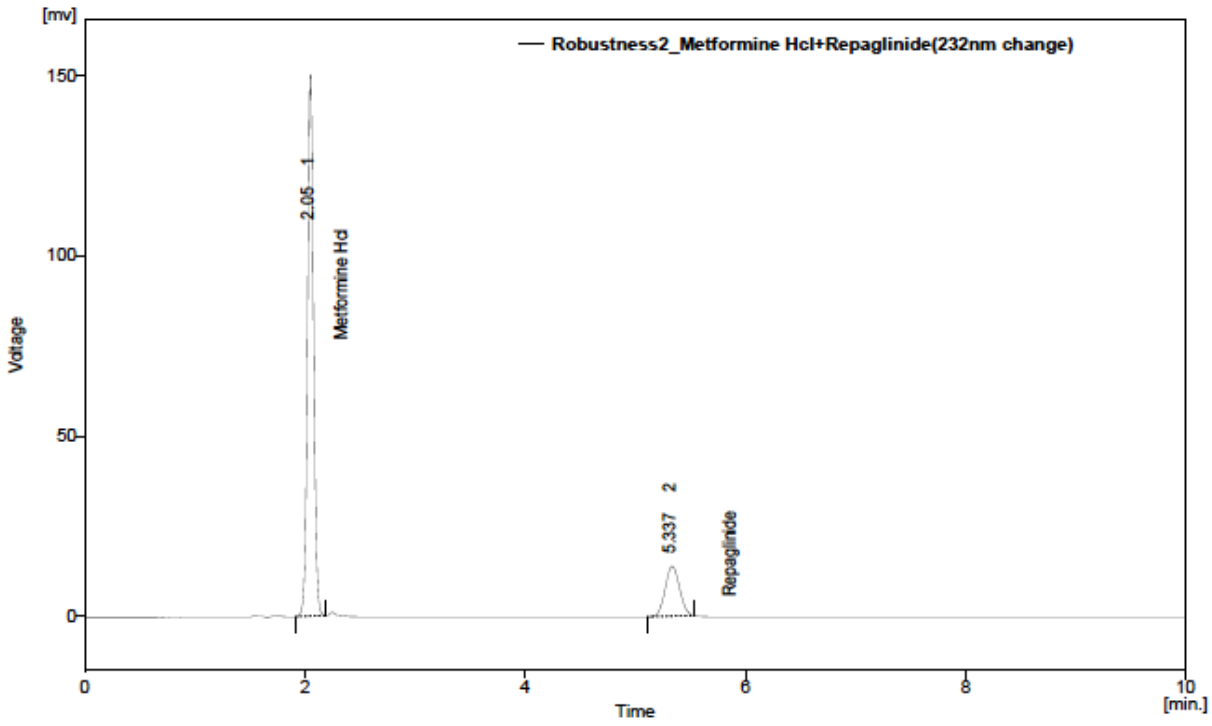
*Column Performance Table (From 50% - Robustness1_Metformine Hcl+Repaglinide(228nm change))*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.057 | 0.060 | 1.125 | 6509 | - |
| 2 | 5.437 | 0.150 | 1.077 | 7278 | 18.942 |

**FIGURE – 45**

**ROBUSTNESS CHROMATOGRAM– II**

**METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg) AT 232 nm**




*Result Table (Uncal - Robustness2_Metformine Hcl+Repaglinide(232nm change))*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.050 | 567.432 | 150.281 | 81.816 |
| 2 | 5.337 | 126.115 | 13.897 | 18.184 |
| | Total | 693.547 | 164.177 | 100.000 |

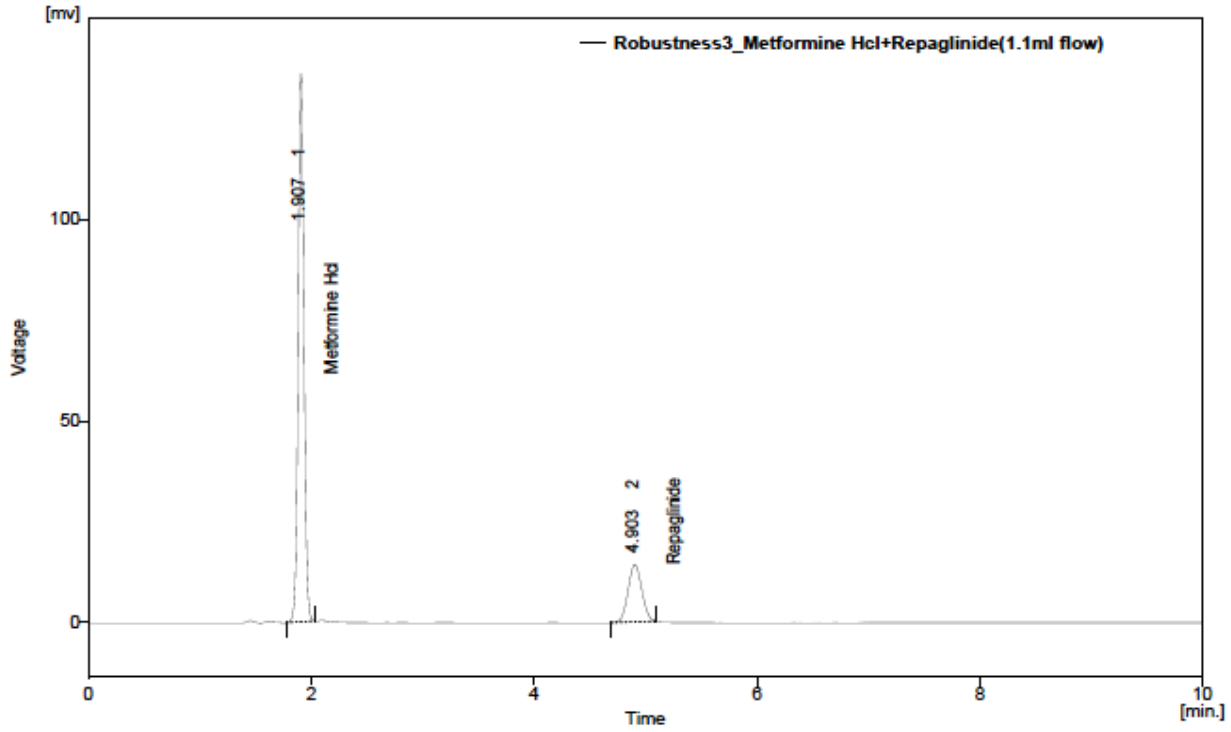
*Column Performance Table (From 50% - Robustness2_Metformine Hcl+Repaglinide(232nm change))*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.050 | 0.060 | 1.125 | 6467 | - |
| 2 | 5.337 | 0.147 | 1.079 | 7335 | 18.716 |

**FIGURE – 46**

## ROBUSTNESS CHROMATOGRAM– III

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg) 1.1 ml flow




*Result Table (Uncal - Robustness3_Metformine Hcl+Repaglinide(1.1ml flow))*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 1.907 | 487.609 | 135.823 | 80.073 |
| 2 | 4.903 | 121.350 | 14.219 | 19.927 |
| | Total | 608.959 | 150.042 | 100.000 |

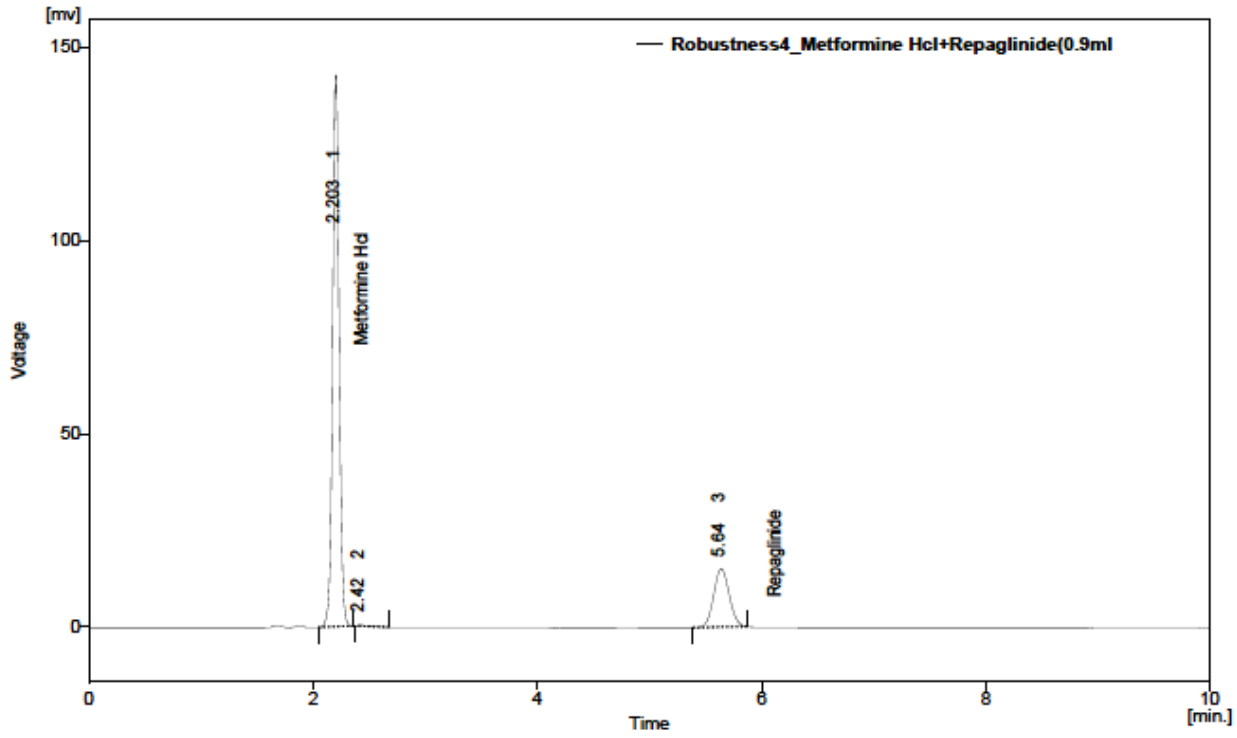
*Column Performance Table (From 50% - Robustness3_Metformine Hcl+Repaglinide(1.1ml flow))*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 1.907 | 0.057 | 1.200 | 6272 | - |
| 2 | 4.903 | 0.137 | 1.114 | 7131 | 18.241 |

**FIGURE – 47**

**ROBUSTNESS CHROMATOGRAM– IV**

**METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg) 0.9 ml flow**




*Result Table (Uncal - Robustness4_Metformine Hcl+Repaglinide(0.9ml)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.203 | 574.699 | 142.845 | 79.444 |
| 2 | 2.420 | 3.078 | 0.528 | 0.425 |
| 3 | 5.640 | 145.622 | 15.138 | 20.130 |
| | Total | 723.398 | 158.512 | 100.000 |

*Column Performance Table (From 50% - Robustness4_Metformine Hcl+Repaglinide(0.9ml)*

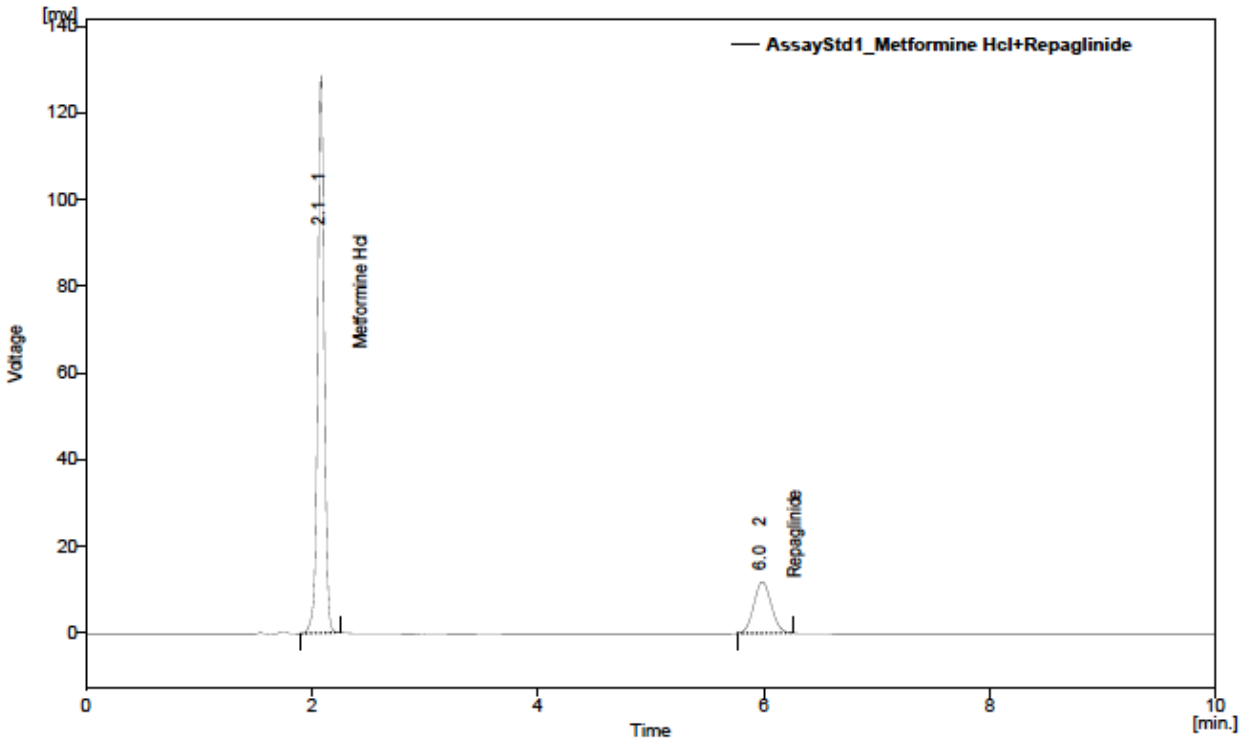
| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.203 | 0.067 | 1.056 | 6051 | - |
| 2 | 2.420 | 0.053 | 4.250 | 11406 | 2.125 |
| 3 | 5.640 | 0.153 | 1.125 | 7495 | 18.336 |

## ASSAY

**FIGURE – 48**

## ASSAY CHROMATOGRAM FOR STANDARD – I

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - AssayStd1_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.080 | 512.476 | 128.487 | 80.114 |
| 2 | 5.987 | 127.206 | 11.848 | 19.886 |
| | Total | 639.682 | 140.335 | 100.000 |

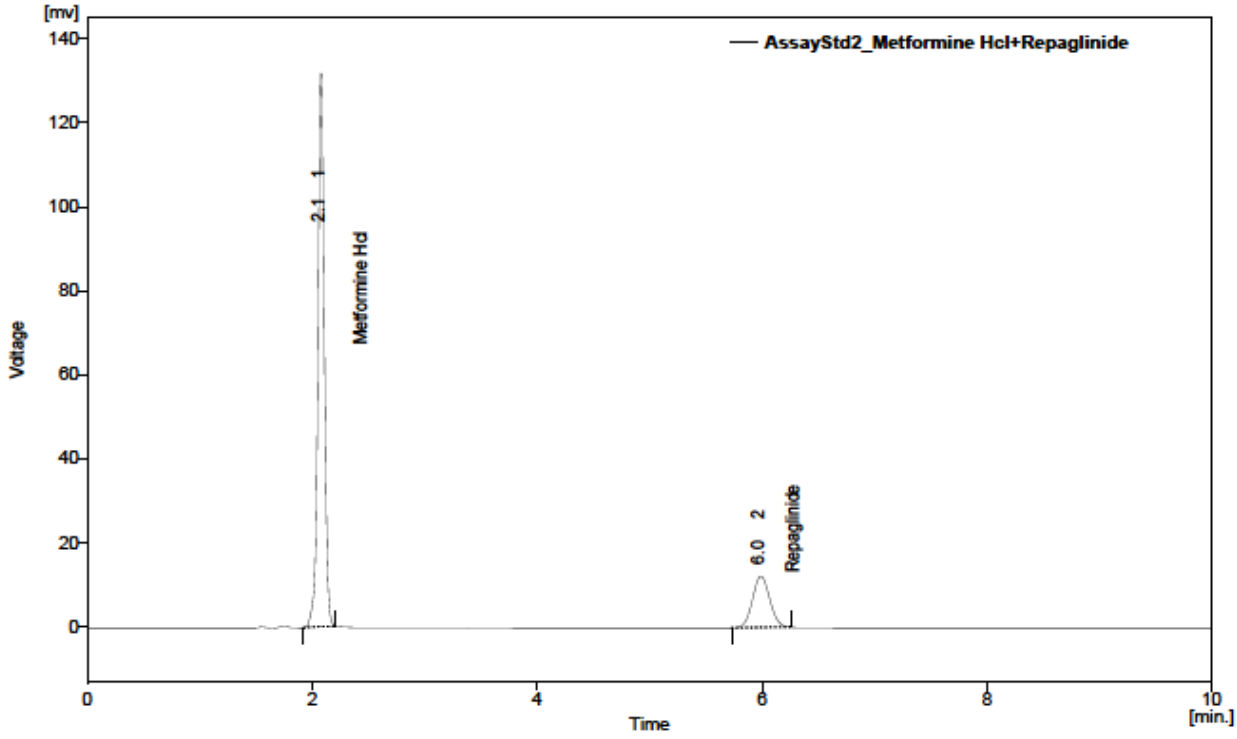
*Column Performance Table (From 50% - AssayStd1_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.080 | 0.063 | 1.118 | 5975 | - |
| 2 | 5.987 | 0.173 | 1.136 | 6609 | 19.426 |

**FIGURE – 49**

## ASSAY CHROMATOGRAM FOR STANDARD– II

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - AssayStd2_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.077 | 517.179 | 131.530 | 79.980 |
| 2 | 5.990 | 129.457 | 12.203 | 20.020 |
| | Total | 646.636 | 143.734 | 100.000 |

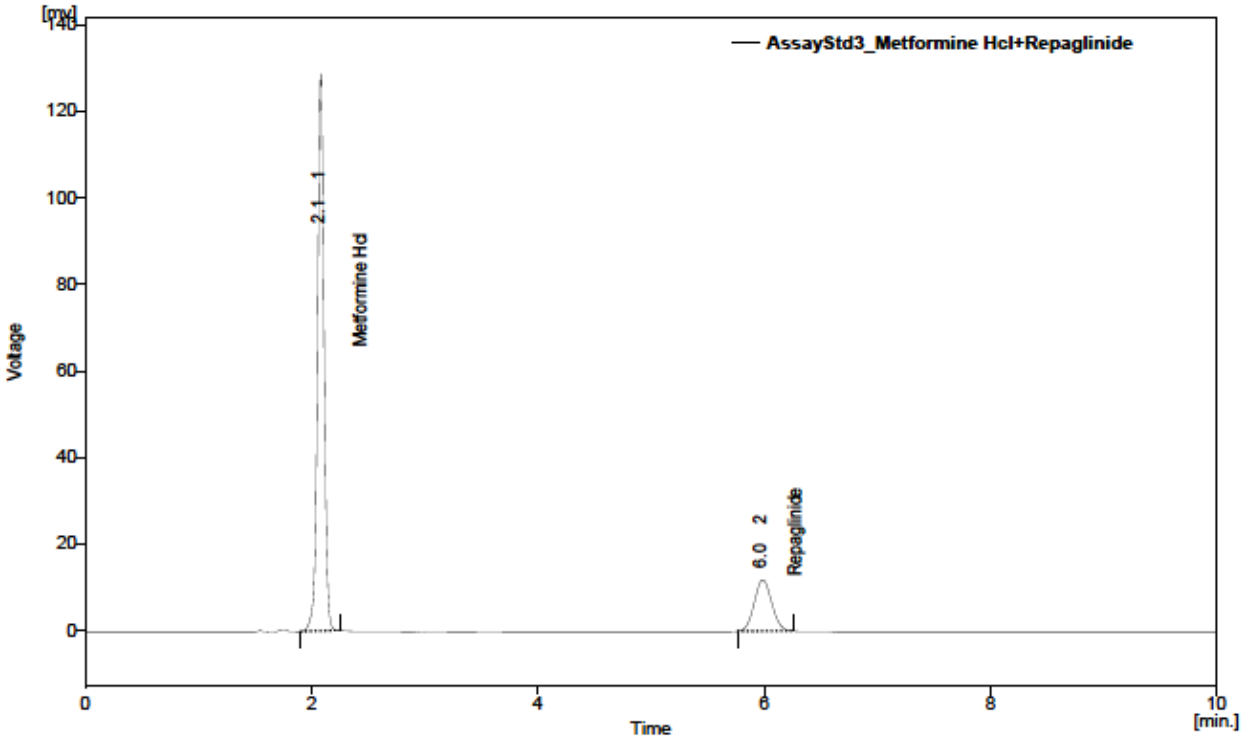
*Column Performance Table (From 50% - AssayStd2_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.077 | 0.060 | 1.118 | 6637 | - |
| 2 | 5.990 | 0.170 | 1.114 | 6878 | 20.024 |

**FIGURE – 50**

## ASSAY CHROMATOGRAM FOR STANDARD– III

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - AssayStd1_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.080 | 514.472 | 128.487 | 80.114 |
| 2 | 5.987 | 128.260 | 11.848 | 19.886 |
| | Total | 642.732 | 140.335 | 100.000 |

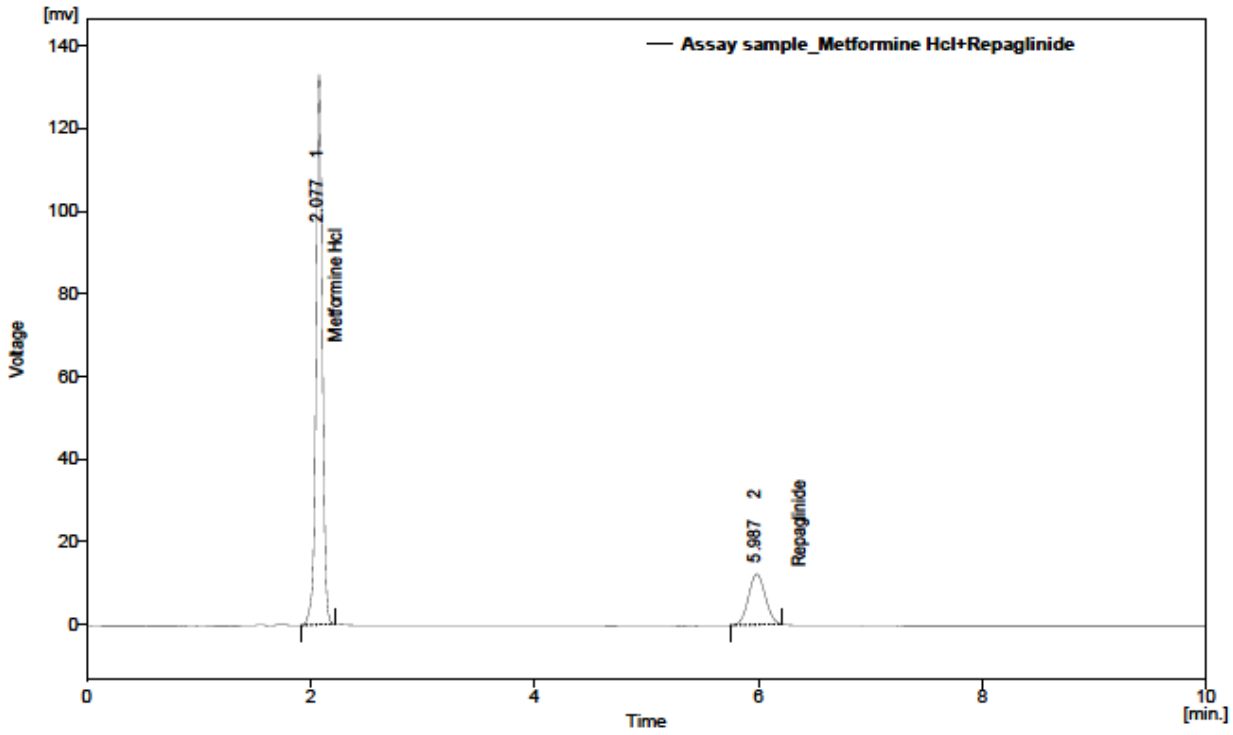
*Column Performance Table (From 50% - AssayStd1_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.080 | 0.063 | 1.118 | 5975 | - |
| 2 | 5.987 | 0.173 | 1.136 | 6609 | 19.426 |

**FIGURE – 51**

## ASSAY CHROMATOGRAM FOR SAMPLE – I

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - Assay Sample_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.077 | 515.713 | 132.846 | 80.329 |
| 2 | 5.987 | 127.754 | 12.259 | 19.671 |
| | Total | 643.467 | 145.105 | 100.000 |

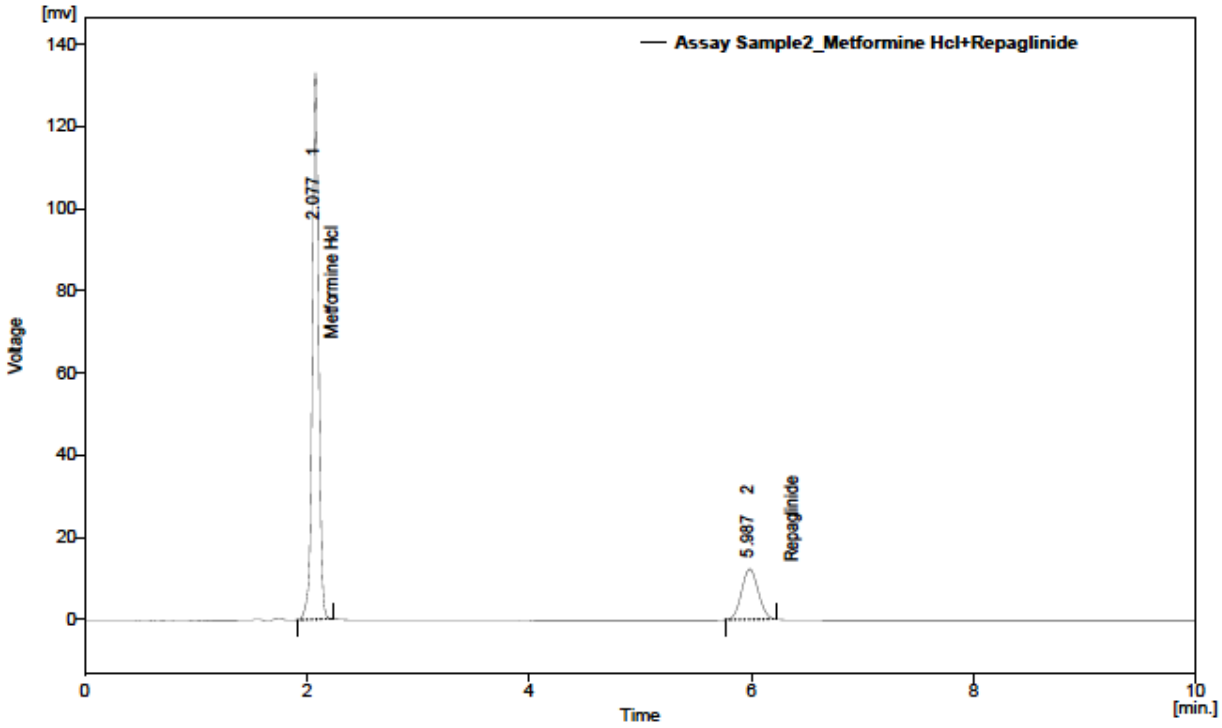
*Column Performance Table (From 50% - Assay Sample_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.077 | 0.063 | 1.059 | 5956 | - |
| 2 | 5.987 | 0.170 | 1.068 | 6870 | 19.721 |

**FIGURE – 52**

## ASSAY CHROMATOGRAM FOR SAMPLE – II

## METFORMIN HYDROCHLORIDE (125 µg) AND REPAGLINIDE (0.5 µg)




*Result Table (Uncal - Assay Sample2_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | Area [mV.s] | Height [mV] | Area [%] |
|---|---|---|---|---|
| 1 | 2.077 | 514.242 | 132.986 | 80.262 |
| 2 | 5.987 | 127.430 | 12.309 | 19.738 |
| | Total | 648.672 | 145.295 | 100.000 |

*Column Performance Table (From 50% - Assay Sample2_Metformine Hcl+Repaglinide)*

| | Reten. Time [min] | W05 [min] | Asymmetry [-] | Efficiency [th.pl] | Resolution [-] |
|---|---|---|---|---|---|
| 1 | 2.077 | 0.063 | 1.059 | 5956 | - |
| 2 | 5.987 | 0.167 | 1.093 | 7148 | 20.007 |

## RESULTS AND DISCUSSION

### SYSYEM SUITABILITY PARAMETERS:

Table no-3: System suitability parameter for Metformine HCl and Repaglinide

| S. no | Parameters | Repaglinide | Metformin hydrochloride |
|---|---|---|---|
| 1. | Resolution | 20.07 | |
| 2. | AUC | 24.459 | 106.384 |
| 3. | No of theoretical plates | 7188 | 5975 |
| 4. | Retention time | 6.003 | 2.060 |
| 5. | Asymmetry | 1.093 | 1.059 |

**Acceptance criteria**: Theoretical plates ≥2000; Retention time ≥ 2; Asymmetry≥2.

**Result**:

Standard solution of Metformine HCl and Repaglinide was determined under proposed condition chromatogram indicating satisfactory % RSD of peak responses, theoretical plates, asymmetry and retention time.

### SPECIFICITY:

**Acceptance criteria**: No interference at the Rt of Metformine HCl and Repaglinide.

**Result:** From the specificity performed, various degradation products are formed and there is no change in the detection of the analyte in the presence of other components.

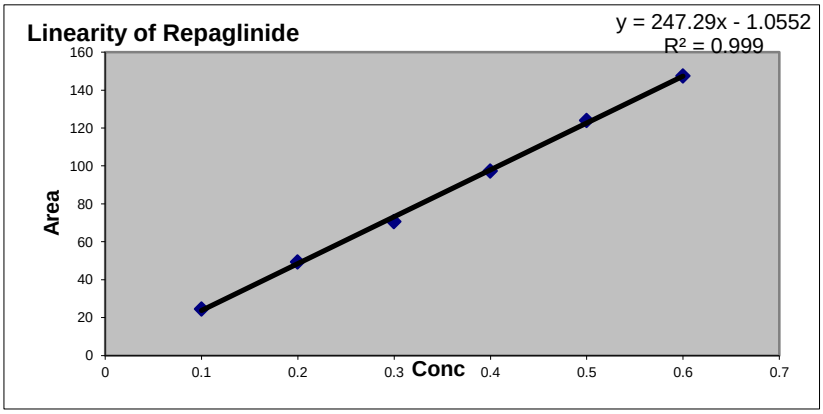
**LINEARITY:**

**Linearity data for Repaglinide**:

Various concentrations of the Repaglinide was made by diluting stock solution to get the concentration of 0.1 to 0.6 µg. The dilution volumes used and peak area obtained are presented below

**Table 4: Linearity data for Repaglinide**

| S. NO | Concentration µg/ml | Peak Area | Statistical Analysis |
|---|---|---|---|
| 1. | 0.1 | 24.459 | |
| 2. | 0.2 | 49.334 | Slope: 247.29 |
| 3. | 0.3 | 70.556 | |
| 4. | 0.4 | 97.251 | Intercept: 1.0552 |
| 5. | 0.5 | 123.888 | |
| 6. | 0.6 | 147.491 | Correlation coefficient: 0.999 |




**Calculation for ppm:**

$$\frac{\text{Std wt x 1x 1000}}{\text{100 x 100}}$$

$$\frac{\text{1 x 1 x 1000}}{\text{100 x 100}} = \text{0.1 ppm}$$

$$\frac{\text{1 x 2 x 1000}}{\text{100 x 100}} = \text{0.2 ppm}$$

$$\frac{1 \times 3 \times 1000}{100 \times 100} = 0.3 \text{ ppm}$$

$$\frac{1 \times 4 \times 1000}{100 \times 100} = 0.4 \text{ ppm}$$

$$\frac{1 \times 5 \times 1000}{100 \times 100} = 0.5 \text{ ppm}$$

$$\frac{1 \times 6 \times 1000}{100 \times 100} = 0.6 \text{ ppm}$$

**Acceptance criteria:** $r^2$ should not be less than 0.99

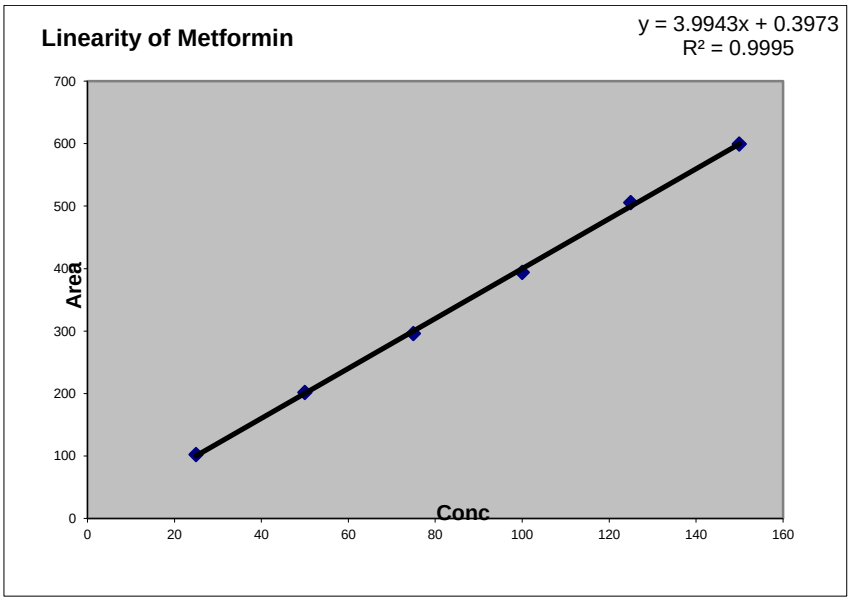
**Result:** The relationship between the concentration and the peak response of Repaglinide was linear in the specific range and the regression coefficient was found to be 0.999.

**Linearity data for Metformin HCl**:

Various concentrations of the metformin HCl was made by diluting stock solution to get the concentration of 25 to 150 µg. The dilution volumes used and peak area obtained are presented below

**Table 5: Linearity data for Metformin HCl**

| S,. no | Concentration µg/ml | Peak Area | Statistical Analysis |
|---|---|---|---|
| 1. | 25 | 102.384 | |
| 2. | 50 | 201.721 | Slope: 3.9943 |
| 3. | 75 | 296.203 | |
| 4. | 100 | 393.97 | Intercept: 0.3973 |
| 5. | 125 | 505.63 | |
| 6. | 150 | 599.49 | Correlation coefficient: 0.9995 |




**Calculation for ppm:**

$$\frac{\text{Std wt} \times 1 \times 1000}{100 \times 100}$$

$$\frac{250 \times 1 \times 1000}{100 \times 100} = 25 \text{ ppm}$$

$$\frac{250 \times 2 \times 1000}{100 \times 100} = 50 \text{ ppm}$$

$$\frac{250 \times 3 \times 1000}{100 \times 100} = 75 \text{ ppm}$$

$$\frac{250 \times 4 \times 1000}{100 \times 100} = 100 \text{ ppm}$$

$$\frac{250 \times 5 \times 1000}{100 \times 100} = 125 \text{ ppm}$$

$$\frac{250 \times 6 \times 1000}{100 \times 100} = 150 \text{ ppm}$$

**Acceptance criteria:** $r^2$ should not be less than 0.99

**Result:** The relationship between the concentration and the peak response of Repaglinide was linear in the specific range and the regression coefficient was found to be 0.9995.

**ACCURACY**

**Accuracy studies for repaglinide:**

Accuracy for the Repaglinide was carried out at three different levels. The recovery data for repaglinide is shown in table below.

**1) Recovery data for repaglinide (0.45mcg):**

| S.no | Concentration(mcg) | Area |
|---|---|---|
| 1 | 0.45 | 115.662 |
| 2 | 0.45 | 114.275 |
| 3 | 0.45 | 114.62 |
| Average area | | 114.8523 |

**Calculation:**

$$\text{Concentration of formulation} = \frac{114.4013 \times 0.5}{128.694}$$

$$= 0.446 \text{ mcg}$$

$$\% \text{ Recovery} = \frac{0.446 \times 100}{0.45}$$

$$= 99.16\%$$

**2) Recovery data for repaglinide (0.55mcg):**

| S.no | Concentration(mcg) | Area |
|---|---|---|
| 1 | 0.55 | 140.278 |
| 2 | 0.55 | 140.101 |
| 3 | 0.55 | 143.825 |
| Average area | | 141.4013 |

**Calculation:**

Concentration of formulation $= \frac{141.4013 \times 0.5}{128.6947}$

$= 0.549$ mcg

Percentage of recovery $= \frac{0.549 \times 100}{0.55}$

$= 99.88\%$

**3) Recovery data for repaglinide (0.65mcg):**

| S.no | Concentration(mcg) | Area |
|---|---|---|
| 1 | 0.65 | 169.913 |
| 2 | 0.65 | 168.128 |
| 3 | 0.65 | 167.945 |
| Average area | | 167.945 |

**Calculation:**

Concentration of formulation $= \frac{167.94 \times 0.5}{128.694}$

$= 0.6524$ mcg

Percentage of recovery $= \frac{0.6524 \times 100}{0.65}$

$= 100.38\%$

**Table 6: Accuracy studies for repaglinide**

| **S.No** | **Mixture of pure and formulation** | **Con. of pure drug, µg/ml** | **Conc. Of Formulation, µg/ml** | **% Recovery of pure drug** |
|---|---|---|---|---|
| 1. | 0.4+0.05 | 0.05 | 0.446 | 99.16 |
| 2. | 0.5+0.05 | 0.05 | 0.549 | 99.88 |
| 3. | 0.6+0.05 | 0.05 | 0.652 | 100.38 |

**Accuracy studies for Metformin HCl:**

Accuracy for the Metformin HCl was carried out at three different levels. The recovery data for Metformin HCl is shown in table below.

**1) Recovery data for metformin (112.5mcg):**

| S.no | Concentration(mcg) | Area |
|---|---|---|
| 1 | 112.5 | 468.646 |
| 2 | 112.5 | 467.646 |
| 3 | 112.5 | 464.123 |
| Average area | | 466.805 |

**Calculation:**

$$\text{Concentration of formulation} = \frac{\text{area of sample} \times \text{concentration of standard drug}}{\text{Average area of sample}}$$

$$= \frac{466.805 \times 125}{522.188}$$

$$= 111.74 \text{ mcg.}$$

$$\%\ \text{Recovery} = \frac{\text{Concentration of formulation} \times 100}{\text{Concentration of sample}}$$

$$= \frac{111.74 \times 100}{112.5}$$

$$= 99.33\%$$

**2) Recovery data for metformin (137.5mcg):**

| S.NO | Concentration(mcg) | Area |
|---|---|---|
| 1 | 137.5 | 572.031 |
| 2 | 137.5 | 574.675 |
| 3 | 137.5 | 574.602 |
| Average area | | 573.7693 |

**Calculation:**

$$\text{Concentration of formulation} = \frac{573.769 \times 125}{522.188}$$

$$= 137.347 \text{ mcg}$$

Percentage of recovery $= \frac{137.347 \times 100}{137.5}$

$= 99.89\%$

**3) Recovery data for metformin (162.5mcg):**

| S.NO | Concentration(mcg) | Area |
|---|---|---|
| 1 | 162.5 | 678.915 |
| 2 | 162.5 | 678.376 |
| 3 | 162.5 | 677.029 |
| Average area | | 678.1067 |

**Calculation:**

Concentration of formulation $= \frac{678.106 \times 125}{52.188}$

$= 162.323$ mcg

Percentage of recovery $= \frac{162.323 \times 100}{162.5}$

$= 99.9\%$

**Table 7: Accuracy studies for Metformin HCl**

| S.No | Mixture of pure and formulation | Con. of pure drug, µg/ml | Conc. Of Formulation, µg/ml | % Recovery of pure drug |
|---|---|---|---|---|
| 1. | 100+12.5 | 12.5 | 111.74 | 99.33 |
| 2. | 125+12.5 | 12.5 | 137.35 | 99.89 |
| 3. | 150+12.5 | 12.5 | 162.32 | 99.89 |

**Report:** The percentage recovery of Repaglinide ranges from 99.16- 100.38% and Metformin HCl ranges from 99.33- 99.89% which are well within the acceptance criteria of 90-110% showing that there is no interference from excipients for the proposed method.

**ROBUSTNESS**

**Table 8. Effect of flow rate**

| Flow rate | RT of Repaglinide | RT of Metformin Hcl |
|---|---|---|
| 0.9 mL/ min | 2.420 | 5.640 |
| 1.1 mL/ min | 4.903 | 1.907 |

**Table 9. Change in wavelength**

| Wave length | RT of Repaglinide | RT of Metformin Hcl |
|---|---|---|
| 228 nm | 5.437 | 2.057 |
| 232 nm | 5.337 | 2.050 |

**PRECISION**

**Precision:**

The system precision is checked to ensure that the analytical system is working properly. The retention time and area of six determinations is measured and % RSD was calculated and represented below.

**Table 10: Precision data for Metformin HCL and Repaglinide**

| S.no | Metformin HCl | | Repaglinide | |
|---|---|---|---|---|
| | Retention | Area | Retention | Area |
| 1 | 2.113 | 510.122 | 6.007 | 126.374 |
| 2 | 2.08 | 511.699 | 5.987 | 125.187 |
| 3 | 2.077 | 517.179 | 5.99 | 127.642 |
| 4 | 2.113 | 509.128 | 6.007 | 127.376 |
| 5 | 2.08 | 519.359 | 5.98 | 126.796 |
| **avg** | 2.0926 | 513.4974 | 5.9942 | 126.675 |
| **stdev** | 0.018663 | 4.517515 | 0.012235 | 0.967713 |
| **%RSD** | **0.89** | **0.88** | **0.20** | **0.76** |

**Calculation:**

$$\% \text{ RSD for Metformin} = \frac{\text{Standard deviation} \times 100}{\text{Mean}}$$

$$= \frac{0.018663 \times 100}{2.0926}$$

$$= 0.891$$

$$\% \text{ RSD for Repaglinide} = \frac{0.012235 \times 100}{5.9942}$$

$$= 0.2041$$

**Amount of Metformin HCl present in mg:**

$$= \frac{\text{Avg sample area x Std wt x dilution factor xTablet wt taken x Std purity}}{\text{Avg std area x Sample wt x 100}}$$

$$= \frac{513.4974 \times 500 \times 820 \times 99.8}{514.709 \times 819 \times 100}$$

$$= 498.433 \text{ mg}$$

$$\text{Assay in \%} = \frac{498.33 \times 100}{500}$$

$$= 99.68 \%$$

**Amount of Repaglinide present in mg:**

$$= \frac{\text{Avg sample area x Standard wt x dilution factor x Tablet wt taken x Std purity}}{\text{Avg std area x Sample wt x 100}}$$

$$= \frac{126.675 \times 2 \times 820 \times 99.9}{128.307 \times 819 \times 100}$$

= 1.97 mg

$$\text{Assay in \%} = \frac{1.97 \times 100}{2}$$

= 98.68 %

**Report:**

From the precision data of the system, the % RSD for Repaglinide and Metformin HCl were found to be 0.76 and 0.88 respectively. Hence, the precision of the system as found to be well within the acceptance criteria (not less than 2%).

**RUGGEDNESS**

The determination was performed by two different analyst (A-1 & A-2) and the chromatograms were recorded. The % RSD was calculated from the peak area.

**Table 11: Ruggedness for Repagl;inide and Metformin HCl**

| **Analyst** | **Retention time of Repaglinide(min)** | **Retention time of Metformin Hcl(min)** |
|---|---|---|
| Analyst 1 | 5.583 | 2.193 |
| Analyst 2 | 5.583 | 2.193 |

**Report:**

From the ruggedness data, the % RSD of peak area by A-1 & A-2 for Repaglinide and Metformin HCl were found to be 5.588 & 2.193 respectively.

**METHOD PRECISION**

The method precision data for Repaglinide and Metformin HCl were presented in table below.

**Table 12: Method precision for Repaglinide and Metformin HCl**

| S.no | Metformin HCl | | Repaglinide | |
|---|---|---|---|---|
| | **Retention** | **Area** | **Retention** | **Area** |
| 1 | 2.077 | 516.525 | 5.973 | 127.447 |
| 2 | 2.077 | 522.248 | 5.987 | 128.434 |
| 3 | 2.077 | 521.713 | 5.987 | 127.754 |
| 4 | 2.077 | 521.242 | 5.987 | 127.43 |
| 5 | 2.077 | 522.998 | 5.987 | 126.176 |
| **avg** | 2.077 | 520.9452 | 5.9842 | 127.4482 |
| **stdev** | 0 | 2.555731 | 0.006261 | 0.819167 |
| **%RSD** | **0.00** | **0.49** | **0.10** | **0.64** |

Amount of Repaglinide present in mg:

$$= \frac{\text{Avg sample area x Standard wt x dilution factor x Tablet wt taken x Std purity}}{\text{Avg std area x Sample wt x 100}}$$

$$= \frac{520.945 \times 500 \times 820 \times 99.8}{514.709 \times 819 \times 100}$$

$$= 505.66 \text{ mg}$$

$$\text{Assay in \%} = \frac{505.66 \times 100}{500}$$

$$= 101.13\ \%$$

Amount of Repaglinide present in mg:

$$= \frac{\text{Avg sample area} \times \text{Standard wt} \times \text{dilution factor} \times \text{Tablet wt taken} \times \text{Std purity}}{\text{Avg std area} \times \text{Sample wt} \times 100}$$

$$= \frac{127.448 \times 2 \times 820 \times 99.9}{128.307 \times 819 \times 100}$$

$$= 1.98\ \text{mg}$$

$$\text{Assay in \%} = \frac{1.98 \times 100}{2}$$

$$= 99.0\ \%$$

**Report**: From the precision data of the method, the % RSD for Repaglinide and Metformin HCl were found to be 0.64 and 0.49 respectively. Hence, the precision of the method was found to be well within the acceptance criteria (not more than 2%).

**ASSAY**

The assay of the standard and samples were carried out with respect to assay procedure from reported method and the marketed samples was analysed to determine the content of Repaglinide and Metformin HCl.

**Table 13: Assay for Repaglinide and Metformin HCl**

| Drug | injection | Peak area | Amount present | % Assay |
|---|---|---|---|---|
| Repaglinide | 1. | 127.754 | 1.99 | 99.46 |
| | 2. | 127.43 | | |
| Metformin hydrochloride | 1. | 515.713 | 499.87 | 99.97 |
| | 2. | 514.242 | | |

**Calculation for Metformin HCl:**

$$= \frac{\text{Avg sample area x Standard wt x Tablet wt taken x Standard purity}}{\text{Avg std area x Sample wt x 100}}$$

$$= \frac{514.9775 \times 500 \times 820 \times 99.8}{514.709 \times 819 \times 100} = 499.87 \text{ mg}$$

$$\text{Assay \%} = \frac{499.87 \times 100}{500}$$

$$= 99.97 \%$$

**Calculation for Repaglinide:**

$$= \frac{\text{Avg sample area x Standard wt x Tablet wt taken x Standard purity}}{\text{Avg std area x Sample wt x 100}}$$

$$= \frac{127.59 \times 2 \times 820 \times 99.9}{128.3077 \times 819 \times 100} = 1.99 \text{ mg}$$

$$\text{Assay \%} = \frac{1.99 \times 100}{2}$$

$$= 99.5 \%$$

## SUMMARY AND CONCLUSION

A very few analytical methods appeared in the literature for the determination of metformin hydrochloride and repaglinide are generally based HPLC, UV, Spectro fluorimetry. That has been reported for the quantification of metformin hydrochloride and repaglinide.

In the present work, an attempt was made to provide a newer, simple accurate and low cost post column derivatization of spectrophotometric and there derivative method and one HPLC method for the effective quantitative determination of metformin HCL and repaglinide as an active pharmaceutical ingredient as well as in pharmaceutical preparations without the interferences of other constituent in the formulations.

For routine analytical purposes it is always of interest to establish methods capable of analyzing a large number of samples in a short time period with good accuracy and precision. The main purpose of the study was to develop accurate, precise and economic methods for the determination of Metformin HCL and Repaglinide. Spectrophotometric technique, and post column derivatization method were applied without using any prior chemical pretreatment in the presence of the strongly overlapping spectra can generate large amounts of data within a short period of analysis.

An HPLC method is developed and validated for various parameters as per ICH guidelines. The system suitability parameters prove that the proposed method is equally suitable for estimation of Metformine HCL and Repaglinide, the chromatogram for Metformin HCl and Repaglinide were found to be satisfactory on RP-18(2), 250X4.6mm, 5µm column, using mobile phase combination of buffer:

acetonitrile (58:42 v/v) with flow rate of 1.0 ml/min. Both the peaks were found to be symmetrical as found from symmetry factor of 1.01 for Metformin HC l and Repaglinide.

The resolution of the proposed method was found to be satisfactory, with peak showing complete baseline separation. The retention rime for Metformin HCl about 5min, and Repaglinide was about 2min. The proposed system of satisfactory phase and mobile phase was ideally suitable for the estimation as indicated by good number of theoretical plates 5689 per meter for Metformin HCl and Repaglinide.

The sensitivity of the method is good and also linearity which is observed is good.

The accuracy of the method is determined by recovery with spiked concentration of pure drug at three levels for metformin HCl and Repaglinide. The recovery of drug is well within the acceptance limits of 97-103%.

The method is rugged and robust as observed from insignificant variation in the results of analysis on changes in mobile phase composition ratio, pH, flow rate, temperature and analysis being performed by different analysts and on different days respectively. In the all above cases the recovery is found to be within the limit.

## CONCLUSION

An HPLC method was developed and validated successfully for simultaneous estimation of Repaglinide and Metformin HCl in tablet dosage form. The present study was validated as per the ICH guidelines and the method was found to be accurate, precise, linear, specific and reproducible for the simultaneous determination of Repaglinide and Metformin HCl in formulation.

From the comprehensive validation conducted, it was concluded that the method is stable and could be used throughout shelf life of the drug.

Hence this study can be extended by studying the degradation kinetics of Repaglinide and Metformin HCl determination by RP-HPLC method and also its estimation in plasma and biological fluids.

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